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THE ANALYST

THE JOURNAL OF THE

Society of Public Analysts and Other Analytical Chemists

A MONTHLY JOURNAL DEVOTED TO THE ADVANCEMENT
OF ANALYTICAL CHEMISTRY

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 7 p.m. on Wednesday, December 1st, 1948, in the Lecture Room of the Institution of Civil Engineers, Great George Street, Westminster, London, S.W.1. The President, Mr. Lewis Eynon, was in the chair.

The general subject of the meeting was "Food Standards and Labelling," and the following contributions were given:—"Introduction," by C. A. Adams, C.B.E., B.Sc., F.R.I.C., Barrister-at-Law; "The View-point of the Manufacturer," by L. H. Lampitt, D.Sc., F.R.I.C., M.I.Chem.E.; "The View-point of the Public Analyst," by H. E. Monk, B.Sc., F.R.I.C.; a general discussion followed.

NEW MEMBERS

James Cassidy, A.R.I.C.; Geoffrey Bernard Courtier, B.Sc. (Lond.), D.I.C., F.R.I.C.; Hugh Francis Edward Donnelly, B.A., B.Sc. (Oxon.), A.R.I.C.; Kenneth Arthur Lees; John Thomas Martin, B.Sc. (Birm.), D.Sc. (Lond.), F.R.I.C.; Nanduri Sambasiva Rao, M.Sc. (Andra Univ.), A.R.I.C.; Wilfred Ebenezer White; Leslie Henry Williams, A.M.C.T., A.R.I.C.

PHYSICAL METHODS GROUP

THE Fourth Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 30th, 1948, in the Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7. Dr. J. G. A. Griffiths, the Chairman of the Group, presided. The Group Officers and elected Members of Committee for the forthcoming year are as follows:—*Chairman*—Dr. J. G. A. Griffiths. *Vice-Chairman*—Mr. B. S. Cooper. *Hon. Secretary*—Dr. J. E. Page, Glaxo Laboratories Ltd., Greenford, Middlesex. *Members of Committee*—Messrs. W. Cule Davies, W. F. Elvidge, L. A. Haddock, R. A. C. Isbell, J. A. C. McClelland and H. E. Monk. *Hon. Auditors*—Messrs. C. A. Bassett and D. C. Garratt (re-appointed).

Votes of thanks were accorded to the three retiring members of Committee, Messrs. J. R. Edisbury, J. Haslam and D. M. Smith, for their services to the Group during the past two years, to the Hon. Auditors and to the Hon. Secretary for their work during the past year.

The Annual General Meeting was followed by the Seventeenth Ordinary Meeting of the Group, at which Mr. R. Donaldson, M.A., of the National Physical Laboratory, delivered a lecture on "The Measurement of Colour."

BIOLOGICAL METHODS GROUP

THE Fourth Annual General Meeting of the Group was held at 6.15 p.m. on Friday, December 17th, 1948, at Gas Industry House, Grosvenor Place, London, S.W.1. At this meeting Dr. E. C. Wood, who had served the Group as Hon. Secretary since its inception, resigned this post, and Mr. S. A. Price was elected to succeed him. The Group Officers and elected Members of Committee for the forthcoming year are as follows:—*Chairman*—Dr. A. J. Amos. *Vice-Chairman*—Mr. N. T. Gridgeman. *Hon. Secretary*—Mr. S. A. Price, The Research Laboratories, Vitamins Ltd., 23 Upper Mall, London, W.6. *Elected Committee Members*—Messrs. A. L. Bacharach, W. A. Broom, H. O. J. Collier, R. L. Edwards, H. E. Monk and H. Pritchard. *Hon. Auditors*—Messrs. D. M. Freeland and J. H. Hamence (as last year).

The Annual General Meeting was followed by an Ordinary Meeting at which the following papers were read and discussed: "The Assay of Aneurine by the Plate Method," by Miss A. Jones and Dr. S. Morris; "The Extraction of Growth Factors from Natural Products prior to Microbiological Assay," by Mr. J. S. Harrison.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held in Manchester on Saturday, December 4th, 1948. The Chairman of the Section, Mr. C. H. Manley, presided, and the attendance was 37. A film, depicting incidents at the Summer Meeting at Windermere, was shown by Mr. J. F. Clark. The following paper was read and discussed: "The Determination of the Meat Content of Sausages," by R. W. Sutton, B.Sc., F.R.I.C., and J. Markland, B.Sc., F.R.I.C.

The Twenty-fourth Annual General Meeting of the Section was held in Manchester on Saturday, January 29th, 1949. The Chairman, Mr. C. H. Manley, presided over an attendance of 30. The Hon. Secretary presented the Report and Financial Statement, which were adopted. Appointments for the forthcoming year were made as follows:—*Chairman*—Mr. J. G. Sherratt. *Vice-Chairman*—Mr. A. A. D. Comrie. *Hon. Secretary*—Mr. Arnold Lees, 87 Marshside Road, Southport, Lancs. *Elected Committee Members*—Messrs. J. F. Clark, C. H. Manley, J. Markland, R. K. Matthews, J. E. Sands and H. Weatherall. *Hon. Auditors*—Messrs. C. J. House and J. R. Walmsley.

The retiring Chairman, Mr. C. H. Manley, presented his "Chairman's Address," after which a number of members contributed to a discussion on several matters of professional interest.

SCOTTISH SECTION

THE Fourteenth Annual General Meeting of the Section was held in Glasgow on January 27th, 1949, and the following office bearers were elected for the coming year:—*Chairman*—Dr. J. Sword. *Vice-Chairman*—Mr. H. C. Moir. *Hon. Secretary and Treasurer*—Mr. R. S. Watson, City Analyst's Department, 20 Trongate, Glasgow, C.1. *Elected Committee Members*—Messrs. F. J. Elliott, A. N. Harrow, M. Herd, J. Malcolm, R. O. Scott and R. G. Thin. *Hon. Auditors*—Messrs. A. R. Campbell and W. M. Cameron.

MICROCHEMISTRY GROUP

THE Fifth Annual General Meeting of the Group was held on Friday, January 29th, 1949, at the Imperial College of Science and Technology, South Kensington, London, S.W.7. It was reported that the number of members of the Group is now 258, an increase of 48 during 1948. The following Officers and Committee Members were elected for 1949:—*Chairman*—Mr. Ronald Belcher. *Vice-Chairman*—Dr. Cecil L. Wilson. *Hon. Secretary*—Mr. Donald F. Phillips. *Hon. Treasurer*—Mr. Clifford Whalley. *Elected Committee Members*—Messrs. A. F. Colson, A. E. Heron, G. Ingram, C. E. Spooner, D. W. Wilson and G. H. Wyatt.

A paper entitled "The Rapid Micro-analytical Determination of Carbon and Hydrogen in Organic Compounds" was read by Dr. A. F. Colson. This was followed by an open discussion on B.I.O.S. Final Report No. 1606: "Progress in Microchemistry in Germany."

Analytical Methods Committee

Tragacanth Sub-Committee

REPORT No. 2

The Evaluation of Flake Tragacanth

THE Analytical Methods Committee has received the following Report from the Tragacanth Sub-Committee and its publication has been duly authorised.

The Sub-Committee is composed as follows:—

Norman Evers, B.Sc., Ph.D., F.R.I.C. (*Chairman*).
D. C. Garratt, B.Sc., Ph.D., F.R.I.C.
C. A. Hallas, B.Sc., F.R.I.C.
J. S. Hamburger (*Hon. Secretary*).
R. S. Higginbotham, B.Sc.
R. H. Marriott, D.Sc., F.R.I.C.
W. Mitchell, B.Sc., Ph.D., F.R.I.C.
G. A. Mittler, Ph.D., F.R.I.C.
W. M. Seaber, B.Sc., F.R.I.C.
S. G. E. Stevens, B.Sc., F.R.I.C.

INTRODUCTION

In Report No. 1 (THE ANALYST, 1948, **73**, 368–377) a method was given for the evaluation of powdered tragacanth. The present report deals with the application of this method to the flake or ribbon gum and with the determination of ash and volatile acidity.

DETERMINATION OF VISCOSITY

It is known that the process of grinding tragacanth may have the effect of lowering the viscosity of mucilages prepared from it, but as a uniform mucilage suitable for the determination of viscosity cannot be produced from the whole gum within a reasonable time, it was considered necessary to powder the sample for the test. The problem before the Sub-Committee, therefore, was to find a method of grinding the whole gum to a degree of fineness suitable for the preparation of a uniform mucilage with a minimum loss of viscosity.

Preliminary experiments suggested that in order to obtain a satisfactory mucilage, it was necessary to use a powder not coarser than that passing a No. 30 mesh sieve. The rejection of the finer powder made little or no difference to the viscosity of the mucilage.

The first series of tests was conducted on two samples of flake tragacanth "A" and "B". Members were asked to carry out the following methods—

(a) take a 50-g. sample, grind to pass a No. 30 mesh sieve, and carry out the method recommended in Report No. 1 except that the mucilage, after the addition of the water, is allowed to stand for 24 hours before being heated.

(*b*) take a 50-g. sample, grind to pass a No. 60 mesh sieve, and carry out the method recommended in Report No. 1, *i.e.*, without allowing the mucilage to stand for twenty-four hours before the heating.

TABLE I

FLAKE GUM "A"

Member	Method	C_{100}	C_{100} on commercial powder
1	(*a*)	0·53	0·64
	(*b*)	0·54	
2	(*a*)	0·52	0·57
	(*b*)	0·54	
3	(*a*)	0·56	0·63
	(*b*)	0·55	
5	(*a*)	0·57	0·60
	(*b*)	0·52; 0·58	
7	(*a*)	0·57	0·63
	(*b*)	0·61	
9	(*a*)	0·51	0·61
	(*b*)	0·58	
10	—	—	0·58
Mean	(*a*)	0·543	0·609
	(*b*)	0·560	

FLAKE GUM "B"

Member	Method	C_{100}	C_{100} on commercial powder
1	(*a*)	0·22	0·28
	(*b*)	0·23	
2	(*a*)	0·19	0·27
	(*b*)	0·20	
3	(*a*)	0·23	0·29
5	(*a*)	0·21; 0·22	0·27; 0·28
	(*b*)	0·20; 0·24	
7	(*a*)	0·22	0·29
	(*b*)	0·23	
9	(*a*)	0·21	0·27
	(*b*)	0·21	
10	—	—	0·26
Mean	(*a*)	0·213	0·276
	(*b*)	0·218	

The results are shown in Table I. C_{100} indicates the concentration necessary to give a mucilage with an efflux time of 100 seconds in the Redwood viscometer. Parallel tests were also carried out by the method recommended in Report No. 1 on powders from the samples of the same gums ground under commercial conditions to pass a No. 120 mesh sieve.

Conclusions—There would seem to be little or no real difference between the results obtained by the two methods (*a*) and (*b*). The results obtained indicate, however, that grinding the flake in the laboratory produces a powder which, when dispersed in water, possesses a viscosity higher than that of the mucilage produced from the commercially ground material. It was decided that the results were sufficiently encouraging to warrant a more extended trial of method (*a*) but it was found that the 24-hour period of standing before heating was unnecessarily long, and the period was reduced to 1 hour.

A second series of tests of the five samples of flake gum "C," "D," "E," "F" and "G" was therefore carried out by the following method—

RECOMMENDED METHOD—

After sifting out any accompanying powder, grind a representative sample, preferably not less than 50 g., until the whole passes through a No. 30 mesh sieve. The grinding may be effected in a coffee mill or a laboratory disintegrator of the Christy and Norris type or by any other method in which the process of grinding is not prolonged and does not appreciably heat the gum. Determine the moisture in the powder by the method given in Report No. 1. Weigh out a quantity of the powdered gum equivalent to the required weight of dry gum into a dry 500-ml. conical flask and add 5 ml. of 95 per cent. alcohol. Ensure that the gum is completely wetted and dispersed evenly over the inner surface of the flask. Add 195 ml. of cold distilled water as quickly as possible and shake. Allow to stand for 1 hour, swirling frequently. Connect the flask to a reflux condenser and place in a vigorously boiling water-bath, so that the surface of the water is about 1 inch above the surface of the mucilage. Complete the determination by the method as described in Report No. 1.

TABLE II

	C_{100}				
Member	"C"	"D"	"E"	"F"	"G"
1	0·226	0·410	0·440	0·73	0·91
2	0·235	0·440	0·450	0·82	0·94
3	0·246	0·490	0·460	0·81	0·90
5	0·226	0·440	0·400	0·83	0·90
7	0·240 0·236	0·413 0·439	0·452	0·74	0·98
8	0·236	0·370 0·380	0·440	0·82	0·96
9	0·246	0·386	0·448	0·77	0·94
Mean	0·236	0·419	0·441	0·790	0·933

These results are regarded as sufficiently concordant for the method described above to be recommended.

NOTE ON THE CORRECTION OF THE REDWOOD VISCOMETER—

It has been pointed out to the Sub-Committee by Dr. G. Barr* of the National Physical Laboratory that the use of the method of correction recommended in Report No. 1 for Redwood Viscometers giving different rates of flow with water may lead to serious errors. The correction applied by the Sub-Committee's recommended method may amount to 10 per cent. whereas differences between instruments certified by the National Physical Laboratory will rarely amount to 2 per cent. at 100 seconds.

Some of the viscometers used have been checked by determining the efflux time at 20° C. of an aqueous solution containing 72·4 per cent. of glycerol and having an uncorrected specific gravity at 25°/25° C. of 1·1886 ± 0·0001. This solution should have an efflux time at 20° C. of 100 seconds. The instruments tested gave efflux times of 100 ± 1 seconds with this solution.

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* Private communication

SUSPENDING POWER

It should be made clear that, although viscosity is one of the factors that determine the suspending power of gum tragacanth, the latter property is dependent on several other factors. It has been shown from experiments by members of the Sub-Committee that mucilages prepared from different gums and having the same efflux time in the Redwood viscometer vary considerably in their suspending power for bismuth carbonate. Viscosity cannot therefore be regarded as a measure of suspending power. Since suspending power is known to be dependent on the physical properties of the suspended particles, it does not seem to be practicable to prescribe a standard method for its determination. It is recommended therefore that when gum tragacanth is being purchased for use as a suspending agent, the purchaser should carry out a form of test using the concentration of tragacanth normally employed in his process and using all the materials that he desires to suspend.

THE DETERMINATION OF ASH*

The percentage of ash is apparently unrelated to the quality of tragacanth, at any rate within the limits encountered in genuine samples, although a high ash content may indicate crude adulteration of the powdered gum.

Preliminary experiments on the determination of ash by direct ignition gave results that did not show satisfactory agreement. Attention was therefore given to the determination of sulphated ash and better concordance was obtained.

The method recommended is as follows—Weigh accurately about 1 g. of the powdered gum into a platinum dish, and ash at about 300° C., preferably over an Argand burner. Moisten the ash with sulphuric acid (50 per cent. by volume) and heat gently until the sulphuric acid is removed, then more strongly until a white ash is obtained. Again moisten with sulphuric acid and ignite at a bright red heat (about 850° C.). The sulphated ash should be expressed as a percentage of the dry gum.

The results on a sample of flake gum "C" and a sample of powdered gum "H" are given in Table III. They are regarded as sufficiently satisfactory to validate the method described above.

TABLE III

RESULTS FOR THE DETERMINATION OF SULPHATED ASH EXPRESSED AS A PERCENTAGE OF THE DRY GUM

	Sulphated ash %	
Member	Flake gum "C"	Powder "H'
1	—	4·62 4·66 4·66 4·68
2	4·89 4·91	4·41 4·47 4·51 4·57
3	4·67 4·73	4·53 4·45
5	4·93 4·97	4·45 4·49
6	4·93	4·47
7	4·74 4·63 4·64	4·37 4·27
8	4·74 4·74	4·59 4·61
9		4·29 4·32 4·58
Mean	4·793	4·500

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* This method is applicable to powdered tragacanth without further grinding.

THE DETERMINATION OF VOLATILE ACIDITY*

The determination of volatile acidity is of value in detecting adulteration of tragacanth with Karaya gum (also known as Indian tragacanth) from *Sterculia* species and with Gum Ghatti (Indian gum) from *Anogeissus latifolia.*

The method used was that described in "*Methods of Analysis of the Association of Official Agricultural Chemists,*" 6th Edition, 1945, p. 709. The details are as follows—

Treat 1 g. of the whole or powdered sample in a 700-ml. round-bottomed long-necked flask in the cold with 100 ml. of water and 5 ml. of phosphoric acid (85 per cent. by weight) for several hours or until the gum is completely swollen. Boil gently for 2 hours under a reflux condenser. A very small quantity of cellulose substance will remain undissolved. Tragacanth yields a practically colourless solution. Karaya gum gives a pink or rose solution. This reaction may be used as a preliminary test for detection of Karaya gum. Distil the hydrolysed product with steam, using a scrubber to connect the distillation flask with the condenser. Continue the distillation until the distillate amounts to 600 ml. and the acid residue to about 20 ml. To avoid scorching of the residue, do not permit concentration of the contents of the distilling flask to less than 20 ml. Titrate the distillate with 0·1 *N* sodium hydroxide, using 10 drops of phenolphthalein indicator. Correct the results by a blank determination and express as "volatile acidity" the number of ml. of 0·1 *N* sodium hydroxide required to neutralise the volatile acid obtained.

The results on two samples of tragacanth "C" and "G" are given in Table IV. The results are calculated as ml. of 0·1 *N* sodium hydroxide per 1 g. of gum as received.

TABLE IV

ml. of 0·1 *N* sodium hydroxide for 1 g.

Member	Gum "C"	Gum "G"
1	3·55; 3·45	3·50; 3·50
2	3·55	3·85
3	3·60	3·75
5	3·55; 3·75	3·50; 3·70
7	3·60	3·80
9	3·90; 3·75	3·90; 3·10

These figures are regarded as sufficiently concordant to justify recommending the method for adoption. Figures obtained by a member using this method on 28 samples of tragacanth gave a range of 2·3 ml. to 4·0 ml. with a mean of 3·46 ml. of 0·1 *N* sodium hydroxide. One sample of Indian gum (Gum Ghatti) gave 12·75 ml. and a sample of Karaya gum 22·2 ml.

The Determination of Steroids in Urine

By S. L. TOMPSETT

(*Read at a Meeting of the Scottish Section at Glasgow, on November* 28, 1947)

STEROID substances are found in the urine of both sexes and are considered to be related to the steroid hormones elaborated by the suprarenal cortex, ovary and placenta—see Tables I and II†. For the most part these are excreted as water-soluble conjugates with glucuronic and sulphuric acids. As a result urine shows but little hormonal activity until the conjugates have been decomposed by acid hydrolysis. It should be noted that the same set of hormones is produced in both the male and the female, but that the hormonal balance is obviously different. In many conditions due to increased or decreased activity of an organ this balance is altered. In the female, changes occur both during the menstrual cycle and in pregnancy.

The isolation and identification of particular steroids has occupied the attention of many workers. Much of this work has been only qualitative although the object in many cases was to attempt to associate the presence of a particular steroid with a particular condition or disease. On the quantitative side, methods have been worked at for the estimation of

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* This method is applicable to powdered tragacanth without further grinding.

† For the basic ring structure of the steroids and the numbering of their carbon atoms, see Appendix, p. 17. In the formulae α-substituents are denoted by dotted linkages and β-substituents by plain linkages.

TABLE I

ORIGIN OF THE PRINCIPAL STEROID HORMONES

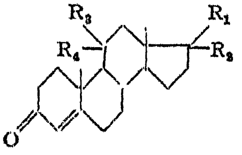
Sex	*Tissue*	*Hormones*
Male	Suprarenal cortex	Corticoids Oestrogens Progesterone Androgens
	Testes	Androgens
Female	Suprarenal cortex	as for male
	Ovary	Oestrogens Progesterone
	Placenta	Oestrogens Progesterone

oestrogens, 17-ketosteroids and pregnanediol. The steroids known to be present in urine may be divided into two groups (see Table III): (1) "phenolic" steroids and (2) neutral steroids. The former contain a phenolic ring in their structure and hence are extractable from solution in organic solvents by aqueous solutions of alkalis. The latter are fully saturated or at least contain no more than one double bond in any 6-membered ring.

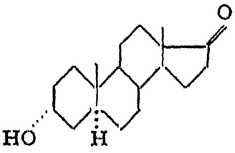
It is probably safe to say that in the past the chemical and physiological study of the steroids has been almost entirely an academic pursuit.

TABLE II

THE PRINCIPAL NATURAL STEROIDS IN MAN



I



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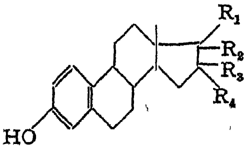


III

(Key formulae)

ANDROGENS—promote male sex activity:

1. Testosterone—I: R_1 = OH; R_2 = R_3 = R_4 = H. The most active; regarded as the hormone.
2. Androsterone—II.

OESTROGENS—promote female sex activity; connected with menstruation, pregnancy and development of the mammae.

1. α-Oestradiol—III: R_1 = OH; R_2 = R_3 = R_4 = H. The most active; regarded as the hormone.
2. Oestrone—III: $R_1 + R_2$ = :O; R_3 = R_4 = H } Probable metabolites of α-oestradiol.
3. Oestriol—III: R_1 = R_3 = OH; R_2 = R_4 = H }

PROGESTERONE—I: R_1 = .$COCH_3$; R_2 = R_3 = R_4 = H. Connected with menstruation and pregnancy.

CORTICOIDS—produced by the suprarenal cortex:

1. Corticosterone—I: R_1 = .$COCH_2OH$; R_3 =OH; R_2 = R_4 = H
2. Desoxycorticosterone—I: R_1 = .$COCH_2OH$; R_2 = R_3 = R_4 = H
3. 11-Dehydrocorticosterone—I: R_1 = .$COCH_2OH$; R_2 = H; $R_3 + R_4$ = :O
4. 17-Hydroxycorticosterone—I: R_1 = .$COCH_2OH$; R_2 = R_3 = OH; R_4 = H
5. 17-Hydroxy-11-dehydrocorticosterone—I: R_1 = .$COCH_2OH$; R_2 = OH; $R_3 + R_4$ = :O
6. 17-Hydroxydesoxycorticosterone—I: R_1 = .$COCH_2OH$; R_2 = OH; R_3 = R_4 = H

The above are concerned with

1. electrolytic balance
2. carbohydrate metabolism
3. ability to combat stress.

TABLE III

CLASSES OF STEROIDS PRESENT IN HUMAN URINE

Phenolic steroids: α-oestradiol, oestrone, oestriol. (See Table II.)
Neutral steroids:
  Ketones:
    17-Ketosteroids. (See Table X.)
      α-Alcoholic, *e.g.*, androsterone
      β-Alcoholic, *e.g.*, *iso*androsterone
      Non-alcoholic, *e.g.*, androstenone-17
    20-Ketosteroids, *e.g.*, allopregnanol-3(β)-one-20. (See Table X.)
    3 : 20-Ketosteroids, *e.g.*, 17-hydroxycorticosterone. (See Table II.)
  Alcohols: (See Table XIII.)
    α-Alcohols, *e.g.*, Pregnanediol-3(α):20(α)
    β-Alcohols, *e.g.*, Androstenetriol-3(β):16:17.

It is now recognised that a study of the urinary steroids is necessary in hospital routine as a diagnostic aid and in the control of certain forms of treatment. Techniques used in hospital biochemical laboratory routine work should conform as far as possible to the following standards. They should—

(1) be as rapid as possible;
(2) permit of more than one estimation being undertaken at the same time;
(3) not require too high a standard of technical skill;
(4) be of an accuracy sufficient for the purpose to which the result is put;
(5) not require too much apparatus, in particular special apparatus difficult to obtain, and take up too much laboratory space.
(6) yield results of clinical value.

During the past 18 months the writer has been carrying out an investigation concerning suitable and useful methods for urinary steroid analysis. The following represents an account of results so far obtained.

HYDROLYTIC PROCEDURES

As a preliminary to any examination, it is necessary to separate the steroids from other materials in the urine. In general three types of procedure have been employed, *viz.*,

(1) extraction of the conjugates with butyl alcohol followed usually by acid hydrolysis,
(2) acid hydrolysis of short duration followed by extraction of the free steroids with an organic solvent,
(3) simultaneous hydrolysis by acid and extraction with a comparatively high-boiling solvent, *e.g.*, toluene or carbon tetrachloride.

There has been much discussion in the literature about the relative merits of various hydrolytic procedures; whatever process is employed, there is some destruction of the steroids, resulting usually in partial desaturation. A completely satisfactory method of hydrolysis is probably an impossibility owing to the large number of different steroids present and the different ways in which they are conjugated.

THE OESTROGENS

Oestrogens are found in male and female urine mainly as water-soluble conjugates. Except in pregnancy the outputs are very low, the measurements being made in micrograms. The oestrogenic content of urine in pregnancy is approximately several hundred times that found in other conditions. (See Table IV.)

TABLE IV

OESTROGENIC CONTENT OF HUMAN URINE (ROBSON[16])

Males	5 to 15 micrograms per day
Females (non-pregnant) ..	10 " 36 " " "
" (pregnant) ..	20,000 " " "

Previous to examination, the urine is hydrolysed with acid and the liberated steroids are extracted with an organic solvent. Owing to their phenolic nature, oestrogens may be separated from the neutral steroids by treatment of the extract with aqueous solutions of alkali.

Numerous colorimetric tests have been suggested for the determination of either total or individual oestrogens (see Table V). These are applicable to the higher concentrations encountered in the urine of pregnancy, but within the more normal range difficulties arise. The main difficulty is the occurrence of relatively high blank values. Where these occur, biological assay, employing ovariectomised rats or mice, is generally used.

TABLE V

CHEMICAL TESTS FOR THE OESTROGENS

	Reactants	Reference
Total oestrogens	Phenol and sulphuric acid	Kober[10] (1931); Venning *et al.*[19] (1937)
	β-Naphthol and sulphuric acid	Kober[11] (1938)
	Sulphuric acid	Cohen and Bates[3] (1947)
Oestrone	*m*-Dinitrobenzene and potassium hydroxide	Talbot *et al.*[17] (1940)
Oestriol	Sodium *p*-phenolsulphonate and phosphoric acid.	Bachman[2] (1939)

There is evidence that deviations from the normal, the measurement of which may be of diagnostic importance, occur in many conditions. Unfortunately such deviations are only measurable in micrograms, as with the normal amounts. Biological assay of the oestrogens is unsatisfactory both in point of accuracy and in the time required—hence a satisfactory chemical method for the determination of minute amounts of the oestrogens is urgently required. It has been found that when oestrogens are injected into human beings only 1 to 10 per cent. can be recovered in the urine as conjugates of α-oestradiol, oestrone and oestriol. The main oestrogen metabolites are therefore unknown. The solution of this problem might lead to more satisfactory methods of following oestrogenic secretion.

THE 17-KETOSTEROIDS

This group, which has received the greatest attention, includes all those neutral steroids that have a ketone group in position 17 (see Appendix). They are found in male and female urine as water-soluble conjugates. Differences between normal and diseased are shown in Table VI. In the main, the free steroids have androgenic, *i.e.*, male sex hormone, activity. In spite of this, however, the variations in the urinary output are in general, representative of adrenal cortical activity.

TABLE VI

THE URINARY EXCRETION OF 17-KETOSTEROIDS—MG. PER DIEM (DORFMAN,[7] 1948)

Normal	Male	8 to 22	average 14
	Female	5 to 15	average 9
On the basis of average figures it is assumed that 9 mg. are derived from the suprarenal cortex and 5 mg. from the testes.			
Addison's disease	Male	up to 5	
	Female	almost 0	
Castration or primary agonadism	Male	9 mg. or less	
	Female	"	
Adreno-genital syndrome	Male	increased excretion; 200 mg. or even more may be encountered	
	Female		
Simmond's disease (panhypopituitarism)	Male	almost 0	
	Female		

NOTE—Low values are encountered in both sexes before puberty and in many chronic diseases.

THE DETERMINATION OF THE TOTAL 17-KETOSTEROIDS IN URINE—

A method has recently been published by Tompsett and Oastler[18] (1946). 250 Ml. of the urine are heated under reflux with 25 ml. of concentrated hydrochloric acid for 10 minutes, after which the liquid is cooled rapidly. Steroids are extracted with peroxide-free ether, and oestrogens, etc., are removed by treating the ether extract with aqueous sodium hydroxide solution followed by water. The ether is removed and the residue dissolved in aldehyde-free alcohol. Two colorimetric procedures:

(1) the Zimmermann reaction,

(2) the Pincus reaction,

are available for the final estimation of the 17-ketosteroid content. In the former, equal parts of the alcoholic solution of steroid, an alcoholic solution of *m*-dinitrobenzene and an aqueous solution of potassium hydroxide are allowed to react at 25° C. for 45 minutes. A purple colour is produced and may be evaluated after suitable dilution with absolute or aqueous alcohol. The test reacts to any compound containing the $-CH_2CO-$ grouping. In the Pincus reaction, the dried steroid residue is heated in a boiling water bath with a solution of antimony trichloride in glacial acetic acid and acetic anhydride. The mixture is diluted with 95 per cent. acetic acid and the colour, which is blue, allowed to develop after standing in the dark for 1 hour at room temperature.

There has been much controversy as to the most suitable hydrolytic and extraction process. Three methods have been compared, *viz.*,

(1) hydrolysis with acid for 10 minutes followed by extraction with ether;

(2) simultaneous hydrolysis with acid and extraction with carbon tetrachloride for 1½ hours;

(3) simultaneous hydrolysis with acid and extraction with toluene for 15 minutes.

All three methods have been found to give similar results when the total 17-ketosteroid content was estimated by the Zimmermann test—the greatest difference was 7 per cent. For single estimations the first procedure has been adopted, but when for any particular purpose large volumes of urine need to be extracted the third procedure is used.

The Zimmermann test is reputed to react more or less equally with all the known urinary 17-ketosteroids. This has been confirmed for the two available 17-ketosteroids, *viz.*, androsterone and dehydro*iso*androsterone. With the Spekker absorptiometer, using green filters and 1-cm. cells, quantities of steroid ranging from 0·00 to 0·20 mg. in 10 ml. gave drum readings (less reagent blanks) ranging, in proportion, from 0·00 to 0·40 for androsterone and from 0·00 to 0·43 for dehydro*iso*androsterone. The Zimmermann test gives but little reaction with the non-ketonic fraction of the urinary steroids.

The Pincus reaction gives somewhat different results. With dehydro*iso*androsterone it produces little colour compared with that given with androsterone. In our own experiments the colour produced by 0·4 mg. of dehydro*iso*androsterone is equivalent to that produced by 0·15 mg. of androsterone. In addition, the non-ketonic fraction of the urinary steroids gives some measurable colour. It has been suggested that comparisons of the results obtained with the two reactions may be a useful indication of the amount of dehydro*iso*androsterone present, but this would, strictly speaking, necessitate a preliminary separation of ketonic and non-ketonic steroids. Whereas the Zimmermann reaction follows Beer's law down to very low levels, the Pincus reaction does not do so below 0·1 mg. of androsterone. With the Spekker absorptiometer, using red filters and 1-cm. cells, the working graph for quantities of androsterone from 0·4 mg. down to 0·1 mg. in 10 ml. was a straight line with drum readings from 0·465 down to 0·075; for quantities of androsterone below 0·1 mg. the graph proceeds to the origin at a reduced slope. Recent work by Jaffe *et al.*[9] (1947) indicates that the Pincus reaction depends on the presence of hydroxyl rather than ketone groupings.

Results obtained by the two reactions on the same urinary steroid extract have been compared (see Table VII). The Zimmermann reaction tends to give slightly higher results, and on general considerations it has been adopted.

TABLE VII

THE 17-KETOSTEROID CONTENT OF URINE—MG. PER DIEM

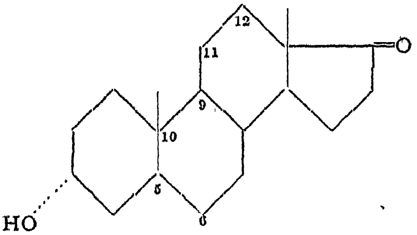
Zimmermann reaction	Pincus reaction
5·9	4·2
3·2	3·8
8·0	7·5
112·0	112·0
8·7	6·1
3·2	2·1

THE DETERMINATION OF α- AND β-ALCOHOLIC AND NON-ALCOHOLIC 17-KETOSTEROIDS IN URINE—

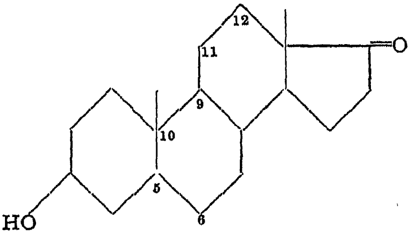
The individual 17-ketosteroids that have been isolated from urine are shown in Table VIII.

TABLE VIII

17-KETOSTEROIDS THAT HAVE BEEN ISOLATED FROM URINE



I



II

(Key formulae)

α-ALCOHOLIC KETONES—not precipitable by digitonin:

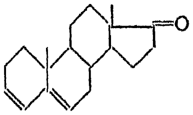
Androsterone, *cis*-Androsterone, Androstane-3(α)-ol-17-one— **I**: α-H at C_5
Actiocholane-3(α)-ol-17-one, 3(α)-Hydroxycholane-17-one— **I**: β-H at C_5
Androstane-3(α):11-diol-17-dione, 11-Hydroxyandrosterone— **I**: α-H at C_5; OH at C_{11}
Δ^{11}-Androstene-3(α)-ol-17-one— **I**: α-H at C_5; double bond at C_{11}-C_{12}
Actiocholanol-3(α)-dione-11:17— **I**: β-H at C_5; :O at C_{11}
Δ^{9}-Actiocholanol-3(α)-one-17— **I**: β-H at C_5; no CH_3 at C_{10} but double bond at C_9–C_{10}

β-ALCOHOLIC KETONES—precipitable by digitonin:

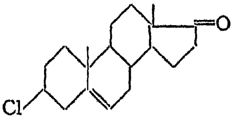
*Iso*androsterone, *Trans*androsterone, Androstane-3(β)-ol-17-one— **II**: α-H at C_5
Dehydro*iso*androsterone, *Trans*dehydroandrosterone, Δ^{5}-Androstene-3(β)-ol-17-one— **II**: double bond at C_5–C_6

NON-ALCOHOLIC KETONES—

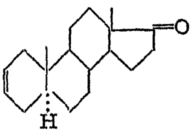
$\Delta^{3,5}$-Androstadiene-17-one—



3-Chlorodehydroandrosterone—



Androstenone-17—



REFERENCES—Pincus and Pearlman[15] (1943), Dobriner *et al.*[6] (1947), Mason[13] (1945), Liebermann and Dobriner[12] (1946), Hirschmann and Hirschmann[8] (1947), Mason and Kepler(1947).[14]

It will be noted that they are divided into three groups, *viz.:*

(1) α-alcohols;
(2) β-alcohols;
(3) non-alcohols.

The non-alcohols, which usually constitute about 15 per cent. of the total, are in all probability derived from the alcohols owing to the destructive effects of acid hydrolysis. This is especially likely since ketosteroids cannot be extracted from urine with organic solvents prior to acid hydrolysis. The α- and β-alcohols represent those with a hydroxyl group attached to the carbon atom 3 and are distinguishable by the fact that the latter but not the former are precipitated from alcoholic solution by digitonin. Normally the β-fraction constitutes about 10 per cent. of the total. High 17-ketosteroid excretion is characteristic of both adrenal cortical hyperplasia and adrenal cortical tumour. There is evidence that in the former, the percentage of β-alcohols is unchanged, but in the latter is much increased as compared with the normal. This is obviously a useful diagnostic test.

For the differential determination of urinary 17-ketosteroids, the following procedure has been found to be satisfactory. Three separate determinations are required:

1. The total 17-ketosteroid content.
2. The non-alcoholic 17-ketosteroid content.
3. The α- and non-alcoholic 17-ketosteroid content after precipitation of the β-alcohols with digitonin.

Then—

Non-alcoholic ketosteroids = 2
α-Ketosteroids = 3 minus 2
β-Ketosteroids = 1 minus 3

NON-ALCOHOLIC KETOSTEROIDS—

An alcoholic solution of urinary steroids is evaporated to dryness in an 8 in. by 1 in. Pyrex test tube. An amount corresponding to about 10 mg. of total 17-ketosteroid should be taken. To the dry residue are added 0·5 g. of succinic anhydride and 2 ml. of pyridine and the tube is plugged with cotton wool. The tube and its contents are placed in a boiling water bath for 2 hours. Then 10 ml. of water are added and the heating is continued for a further ½-hour. After cooling, the contents of the tube are extracted 3 times with 15-ml. portions of ether. The ether extract contains the alcohols as half succinate esters and the non-alcohols unchanged. The ether solution is then extracted with three 20-ml. portions of 0·1 *N* potassium carbonate in 5 per cent. sodium chloride solution. The esterified alcoholic ketosteroids are removed by this process, the non-alcoholic ketosteroids being left in the ether. The ether solution is washed twice with 10 ml. of water and evaporated to dryness. The residue is dissolved in 2 ml. of aldehyde-free alcohol and the 17-ketosteroid content determined in 0·2 ml. by the method of Tompsett and Oastler[18] (1946).

Five-mg. and 10-mg. lots of both androsterone and dehydro*iso*androsterone were submitted to this treatment and the residual ketosteroid content of the ether was determined. It amounted to from 0·5 to 3·5 per cent. of the original amount of steroid taken.

ALPHA ALCOHOLIC AND NON-ALCOHOLIC KETOSTEROIDS

An alcoholic solution of urinary steroids containing about 5 mg. of total 17-ketosteroids is transferred to a 50-ml. centrifuge tube. The volume is adjusted to 2 ml. either by evaporation or by dilution with alcohol. Three ml. of a 2 per cent. solution of digitonin in 83 per cent. alcohol are added and the mixture is allowed to stand overnight. Next day, 10 ml. of ether are added and the mixture is centrifuged. The supernatant fluid is removed and the residue treated with three 10-ml. portions of ether. The combined ether extracts, which contain the α-alcohols and the non-alcohols, are washed with three 25-ml. portions of water and then evaporated to dryness. The residue is dissolved in 10 ml. of aldehyde-free alcohol and the 17-ketosteroid content determined in 0·2 ml. according to the method of Tompsett and Oastler.

Mixtures of androsterone and dehydro*iso*androsterone were submitted to the above treatment. The results are shown in Table IX.

TABLE IX

RECOVERY OF ANDROSTERONE FROM A MIXTURE OF ANDROSTERONE AND DEHYDRO*iso*ANDROSTERONE AFTER PRECIPITATION OF THE DEHYDRO*iso*ANDROSTERONE WITH DIGITONIN

Androsterone (mg.)	Dehydro*iso*androsterone (mg.)	Recovery (mg.)
4·5	0·5	4·4
4·0	1·0	3·9
3·0	2·0	2·8

CHROMATOGRAPHIC ANALYSIS

There are indications that individual variations in the quality of the 17-ketosteroids may be of significance in diagnosis, possibly in malignant disease. The isolation of individual ketosteroids is well beyond the scope of the routine laboratory but fluid chromatography has been used by Dobriner *et al.*[5] (1944) and Dingemanse *et al.*[4] (1946). This appears to have possibilities.

THE 3 : 20-KETOSTEROIDS

For some years it has been recognised that extracts of human urine are capable of maintaining the life of adrenalectomised animals in the same way as the normal hormones of the suprarenal cortex. The active substances are extractable by organic solvents without previous hydrolysis; in fact, treatment with acid has a destructive effect. This is the only steroid fraction that is known to be excreted in an unconjugated state. Although none has yet been isolated and identified, they would appear to be 3 : 20-ketosteroids as the cortical hormones. Increased excretions are noted after stresses, *e.g.*, injury, burns and Cushing's disease. Variations in their amount then have a diagnostic importance, especially as they do not necessarily follow the urinary excretion of 17-ketosteroids. This group has been referred to as:

(1) the 11-oxycorticosteroids;
(2) the total reducing ketonic steroids;
(3) the corticoids.

Tompsett and Oastler[18] (1947) have developed a chemical method for their estimation. This involves extraction with chloroform, separation as a Girard-T complex, liberation of the free ketones and a colorimetric determination of their reducing power towards an alkaline copper reagent. Such substances are typified by reducing alkaline copper reagents in the same way as sugar. The 3 : 20-ketosteroid content of normal urine is of the order of 0·2 to 0·4 mg. per day.

PREGNANEDIOL

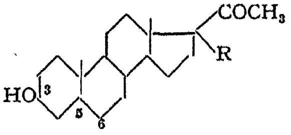
Pregnanediol is the only steroid alcohol that has received much attention. The term "pregnanediol" refers specifically to pregnanediol-3 (α) : 20 (α). It is believed to be a metabolite of progesterone, a hormone elaborated by the corpus luteum of the ovary and the placenta in women and by the suprarenal cortex of both sexes. It is excreted as a water-soluble conjugate with glucuronic acid and is inactive. It occurs in measurable quantity only in female urine. In the non-pregnant female it is excreted in amounts up to 8 mg. per day, depending on the stage of the menstrual cycle. In pregnancy as much as 100 mg. per day may be excreted in the later stages. Tompsett and Oastler[18] (1948) have used a modification of the method of Astwood and Jones[1] (1941). The daily output of urine is simultaneously hydrolysed with hydrochloric acid and extracted with toluene. The toluene extract is washed with aqueous sodium hydroxide solution and then with water and evaporated to dryness The residue is dissolved in 10 ml. of alcohol, 40 ml. of hot 0·1 *N* sodium hydroxide are added and the mixture is allowed to stand overnight in the ice chest. The next morning the precipitate is filtered off. It is dissolved in 10 ml. of alcohol, 40 ml. of hot water are added, and the mixture is allowed to stand overnight in the ice chest. The next morning, the precipitate is filtered off, dried and weighed. This is reckoned as "pregnanediol." This method is based on the observation that under the above conditions pregnanediol is precipitated in filtrable condition, whereas other steroids form colloidal solutions. The estimation seems to have some application in obstetrics and gynaecology.

THE DETERMINATION AND QUANTITATIVE FRACTIONATION OF THE NEUTRAL STEROIDS OF URINE

The quantitative aspects of steroid metabolism, so far as urine is concerned, have been confined mainly to estimations of the 17-ketosteroid, corticosteroid and pregnanediol contents. Isolation studies have shown that other steroids or steroid groups may be present, *e.g.*, 20-ketosteroids and steroid alcohols other than pregnanediol. Members of these series that have been isolated from human urine are shown in Tables X and XI. Attempts have, therefore, been made to determine quantitatively the various steroid fractions that do or might occur in human urine. Since so little information was available, this was largely of an exploratory nature and urines from a wide variety of clinical states were examined. It was

TABLE X

20-KETOSTEROIDS THAT HAVE BEEN ISOLATED FROM HUMAN URINE



(Key formula)

Pregnanol-3(α)-one-20— α-OH at C_3; β-H at C_5; R = H
Allopregnanol-3(β)-one-20— β-OH at C_3; α-H at C_5; R = H
Allopregnanol-3(α)-one-20— α-OH at C_3; α-H at C_5; R = H
Pregnanediol-3(α):17-one-20— α-OH at C_3; β-H at C_5; R = OH
Δ^5-Pregnenediol-3(β):17(β)-one-20— β-OH at C_3; double bond at C_5-C_6; R = OH

REFERENCES—Pincus and Pearlman[15] (1943), Liebermann and Dobriner[12] (1945), Hirschmann and Hirschmann[8] (1947).

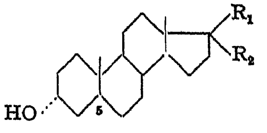
necessary to confine oneself to quantities of urine no greater than that passed during 24 hours, as otherwise important variations might be missed.

The following method was adopted for obtaining crude extracts of the urinary neutral steroids.

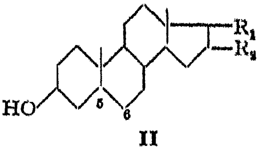
The complete 24-hour sample of urine, together with one-tenth of its volume of redistilled toluene, was heated to boiling under a reflux condenser. Then one-tenth of its volume of concentrated hydrochloric acid was added and the boiling continued for a further 15 minutes. The mixture was cooled and the toluene removed in a separating funnel. The hydrolysed urine was further extracted with two similar quantities of toluene. The combined toluene extracts were washed twice with aqueous 10 per cent. sodium hydroxide solution and twice with water. The toluene extracts were evaporated to dryness, preferably *in vacuo*. The dry residue contained the free steroids, which were invariably mixed with pigment.

TABLE XI

ALCOHOLS THAT HAVE BEEN ISOLATED FROM HUMAN URINE



I



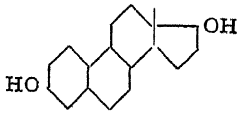
II

(Key formulae)

α-ALCOHOLS—
Pregnane-3(α):17:20-triol— I: β-H at C_5; R_1 = .CHOH.CH$_3$; R_2 = OH
Pregnanediol-3(α):20(α)— I: β-H at C_5; R_1 = .CHOH.CH$_3$; R_2 = H
Allopregnanediol-3(α):20(α)— I: α-H at C_5; R_1 = .CHOH.CH$_3$; R_2 = H
Pregnanol-3 (α)— I: β-H at C_5; R_1 = .CH$_2$CH$_3$; R_2 = H

β-ALCOHOLS—
Cholesterol
Δ^5-Pregnenediol-3(β):20(α)— II: double bond at C_5–C_6; R_1 = .CHOH.CH$_3$; R_2 = H
Δ^5-Androstenediol-3(β):17(α)— II: double bond at C_5–C_6; R_1 = OH; R_2 = H
Allopregnanediol-3(β):20(α)— II : α-H at C_5; R_1 = .CHOH.CH$_3$; R_2 = H
Δ^5-Androstenetriol-3(β):16:17— II: double bond at C_5–C_6; R_1 = R_2 = OH

UNCLASSIFIED—
Oestranediols—



REFERENCES—Pincus and Pearlman[15] (1943), Hirschmann and Hirschmann[8] (1945), Mason and Kepler[14] (1945).

The quantitative separation and determination of the ketonic and non-ketonic steroids was attempted. For this purpose, Girard's reagent T was employed. Under appropriate conditions this reagent forms with ketones, but not with non-ketones, a compound soluble in water but insoluble in organic solvents. Such compounds are, however, readily converted into free ketones in the presence of mineral acids at room temperature.

The dry steroid residue was dissolved in ether and an aliquot containing approximately 10 to 15 mg. of steroid introduced into an 8 in. by 1 in. Pyrex test tube. The solvent was evaporated and 0·1 g. of Girard reagent T (kept in a vacuum desiccator over concentrated sulphuric acid) and 0·5 ml. of glacial acetic acid were added. The tube was plugged with cotton wool and placed in a boiling water bath for 20 minutes. The tube and its contents were cooled in a mixture of ice and water. Thirty ml. of ice-cold water and 3 ml. of 10 per cent. sodium hydroxide solution were added, the latter neutralising nine-tenths of the acetic acid initially present. The mixture was transferred to a 50-ml. glass-stoppered cylinder and the non-ketones were extracted with three 20-ml. quantities of ether. The combined ether extracts were transferred to a separating funnel and washed once with 20 ml. of 0·1 *N* sodium hydroxide and twice with 20-ml. quantities of water. The washed ether extract was transferred to a weighed beaker, the solvent evaporated off and the quantity of non-ketonic material determined gravimetrically.

Three ml. of concentrated hydrochloric acid were added to the aqueous solution remaining and the mixture was allowed to stand overnight at room temperature. On the following day, the freed ketones were extracted with three 20-ml. quantities of ether. The combined ether extracts were washed once with 20 ml. of 0·1 *N* sodium hydroxide and twice with 20-ml. quantities of water. The washed ether extract was transferred to a weighed beaker, the solvent evaporated off and the quantity of ketonic material determined gravimetrically.

Both the ketonic and non-ketonic residues contained pigment. The 17-ketosteroid content was determined by means of the Zimmermann reaction (Tompsett and Oastler[18] (1946)).

Pure ketonic steroids have been subjected to the above treatment and their recoveries are shown in Table XII. In view of the small quantities used, reasonable recoveries would appear to be possible.

TABLE XII

RECOVERY OF KETONIC STEROIDS AFTER SEPARATION AS COMPLEXES WITH GIRARD REAGENT T

	Ketonic steroid used (mg.)	Ketonic steroid recovered (mg.)
Androsterone	5	5·3
	6	5·9
	10	9·6
Dehydro*iso*androsterone..	2·5	2·2
	5·0	4·3
	10·0	9·6

Samples of urine from a wide variety of clinical cases have been examined. Some typical results are shown in Table XIII.

TABLE XIII

THE TOTAL KETONE AND 17-KETOSTEROID CONTENT OF THE NEUTRAL STEROID FRACTION OF HUMAN URINE—SOME TYPICAL RESULTS

	Total ketone neutral steroids (mg./diem)	17-Ketosteroids (mg./diem)
1	2·1	1·5
2	3·1	2·0
3	3·6	3·5
4	10·6	8·3
5	10·0	9·4
6	17·7	16·8
7	19·5	17·5
8*	65·8	8·5
9*	56·0	13·3

* Females—8th month of pregnancy.

In many instances close agreement existed between the 17-ketosteroids and the total ketone content. This would suggest the absence, or extremely low concentrations, of 20-ketosteroids. The chief exception was encountered in urines from cases of late pregnancy.

It would appear that in this condition very large quantities of 20-ketosteroids are excreted. Although pregnanolenes have been isolated from human pregnancy urine, no quantitative results have previously been recorded. Recently Venning (1946) suggested that in late pregnancy approximately 80 mg. of pregnonolone per day was excreted—this was based on a difference in the 17-ketosteroid content as determined by the Zimmermann and Pincus reactions. Dr. Oastler and I have obtained evidence that 20-ketosteroids may be excreted in small quantities in certain other clinical states—these are being recorded elsewhere.

The total ketone and non-ketone contents of urine in some typical cases are shown in Table XIV. It will be noted that the non-ketonic fraction of the neutral steroids of human urine are of the same order of magnitude as the ketonic fraction. Oastler and I have observed significant variations in the magnitude of this fraction in some clinical conditions—these have been recorded elsewhere (Tompsett and Oastler,[18] 1948).

TABLE XIV

THE KETONIC AND NON-KETONIC FRACTIONS OF THE NEUTRAL STEROIDS OF HUMAN URINE

	Total ketones mg./diem.	Non-ketones mg./diem.
1	2·1	5·0
2	3·6	2·4
3	1·2	2·7
4	10·6	7·7
5	14·7	14·0
6	18·5	17·4
7	17·7	23·0
8	25·0	24·0

The nature of the non-ketonic fraction has received some investigation. Steroid alcohols react with succinic anhydride to form half succinate esters. When this reaction was applied to the non-ketonic fraction, it was found that approximately two-thirds reacted to form succinate esters. It is considered therefore that variations in the non-ketonic fraction reflect quantitatively the variations in the alcoholic fraction of the urinary neutral steroids. By application of the well known reaction with acetic anhydride and sulphuric acid, cholesterol was found to be present in only negligible proportions.

Owing to the complexities and inter-relationships of steroid hormone metabolism, estimation of a single substance or group of substances gives only limited information. A more complete picture may be given by the following determinations:

(1) Oestrogens.
(2) 17-ketosteroids.
(3) Total ketones—indicative of presence of the 20-ketosteroids.
(4) The non-ketones.
(5) Pregnanediol-3 (α) : 20 (α).
(6) The corticosteroids.

It is, of course, possible that with increasing knowledge, the number of estimations may be limited in the examination of any particular case. For example, at present it is known that pregnanediol-3 (α) : 20 (α) is related to the metabolism of progesterone and hence has little value in the investigation of the male.

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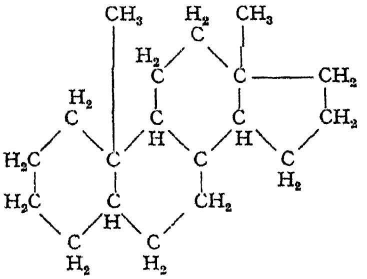
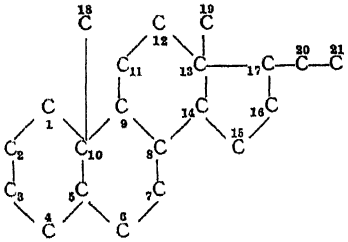
APPENDIX

Basic ring structure of the steroids.

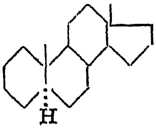


Numbering of the carbon atoms.

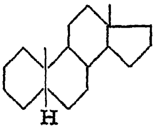


Mother Substances

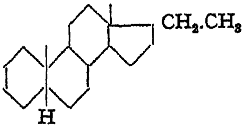
Androstane



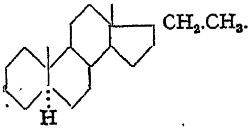
Aetiocholane



Pregnane



Allopregnane



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The Determination of Aneurine (Vitamin B_1) in Uncooked Wheat Products

By H. N. RIDYARD

THE methods described below do not differ essentially from the classical thiochrome method of Jansen,[1] improved by Harris and Wang[2,3] and adapted by Booth[4] (see also Nicholls *et al.*[5]) for use with the Spekker Fluorimeter. They do, however, include a great number of refinements that experience and detailed investigation have shown to be of the utmost importance for the attainment of high accuracy and speed and also for ease of approach for workers new to this estimation. It has been found that junior workers previously unacquainted with aneurine or this kind of work can obtain results to within ± 5 per cent. on National flour with this written account as their only guide.

REAGENTS REQUIRED—

Sodium hydroxide solution, 30 per cent.—30 g. of purest sodium hydroxide dissolved in water with stirring, allowed to cool and diluted to 100 ml.

Potassium ferricyanide solution, 5 per cent.—5 g. of potassium ferricyanide (purest) dissolved in 100 ml. water.

Methyl alcohol, pure, free from fluorescence.

Ethyl alcohol, pure, free from fluorescence, absolute or rectified (96 to 100 per cent).

Iso*butyl alcohol,* pure—This should be steam distilled in all-glass apparatus, using distilled water, the first and last fractions to distil being returned to residues. After use it may be recovered by removing the aqueous layers in a separating funnel, washing three times with distilled water to remove lower alcohols, and steam distilling (see Note 1, page 22). It is then cooled to room temperature, preferably by standing overnight or longer, to deposit excess of water, and is used water-saturated but not cloudy. This and the other reagents should be shown to be free from fluorescence affecting the estimation in the manner described later.

Hydrochloric acid, approximately 0·2 *N*—Twenty ml. of purest hydrochloric acid (36 per cent. w/v) are diluted to 1 litre.

PREPARATION OF EXTRACTS—

(*a*) *Flour*—10 g. of the flour are weighed into a 100-ml. conical flask. 50 ml. of approximately 0·2 *N* hydrochloric acid are placed in a measuring cylinder, from which about 30 ml. are added to the flour and shaken vigorously to form a smooth suspension. The flask may be corked during shaking if the cork is quite clean and kept for this purpose only. The cork and the interior of the flask are washed down with the remainder of the acid and the mixture is allowed to stand overnight.

(*b*) *Wholemeal flour*—5 g. are treated as above.

(*c*) *Bran, etc.*—2 or 3 g. are placed in a 100-ml. flask and 50 ml. of 0·2 *N* acid added without shaking and allowed to stand overnight.

(*d*) *Wheat*—20 g. of whole wheat are weighed and passed through a hand mill (coffee mill) with precautions against contamination or loss. The mill should be prepared for use by grinding a small portion of wheat, turning the mill until no more material is ejected, and rejecting this material. The ground material is placed in a 250-ml. conical flask; 200 ml. of 0·2 *N* hydrochloric acid are measured out, about half is added to the flask, which is then agitated, and the remaining acid is used to wash down the flask, which is allowed to stand overnight (see Note 2).

It is advisable to prepare extracts in duplicate.

In every case the extracts are well shaken the following morning, and allowed to settle, and the liquids poured into centrifuge tubes or test tubes. These are allowed to stand for 1 hour or more to settle, or centrifuged until fairly clear. It is best to pour off bran or wholemeal extracts through a pad of glass wool to remove floating particles, as these may give a high colour with alkali—and so affect the result.

PREPARATION OF STANDARDS—

0·100 g. of pure crystalline aneurine hydrochloride (see Note 3) is weighed in a watch glass, washed through a funnel into a 500-ml. measuring flask with 0·2 *N* hydrochloric acid and made up to 500 ml. with similar acid. Of this solution 25 ml. are taken and made up to 500 ml., giving a solution containing 10 µg. per ml. Quantities of this solution ranging from 5 to 55 ml. in 5 ml. steps are taken and made up to 500 ml., giving a series of standards ranging from 0·1 up to 1·1 µg. per ml. in 0·1 µg. steps. For all the dilutions 0·2 *N* hydrochloric acid is used; the solutions keep well for months in well-stoppered bottles. It is essential to make up other solutions independently for use as checks on accuracy (see Note 4).

Mixing is a serious problem with such dilute solutions; if they are made up in measuring flasks they should be well shaken, then emptied by inverting the flask into a dry beaker, the solution being returned to the flask and again emptied into the beaker, five times in all. Where determinations are a routine it is preferable to find the weight of the 500 ml. of acid, and to weigh all the successive volumes in stoppered tared flasks of about 700 ml. capacity in which the solutions can be shaken thoroughly. Flasks with standard ground necks, tared to the same weight, when unstoppered, by means of a lead strip soldered round the neck, are of great assistance.

PREPARATION OF THE QUININE SOLUTION—

0·08 g. of quinine bisulphate is weighed in a watch glass, washed into a flask and made up to 500 ml. with 0·1 *N* sulphuric acid. Of this solution 5 ml. are diluted to 1000 ml. with the same acid, giving a solution containing 0·8 µg. per ml. If batches of this solution are made up at later dates, they may be adjusted to give the same curve by appropriate addition of 0·1 *N* sulphuric acid or more concentrated quinine solution as necessary, thus compensating for moisture or other variations in the solid quinine bisulphate.

METHOD OF OXIDATION OF EXTRACTS AND STANDARDS—

Test-tubes, 150 × 25 mm., in sufficient number to accommodate all the extracts under examination, together with at least four standards, are placed in a rack, which is conveniently circular, holding about twenty tubes and with accommodation for each tube of extract behind its appropriate test-tube. Two standards, 0·8 µg. per ml., may begin the set, which may be terminated by standards of 0·6 and 0·4 µg. per ml. Two ml. of standard solution or extract are placed in each tube and, when all have been measured, 2 ml. of pure methyl alcohol added to each. While a current of clean air to cause violent agitation is passed into the first tube through a 5-mm. glass tube drawn to a jet, 1 ml. of 30 per cent. caustic soda solution is added, followed about 6 seconds later by 0·25 ml. of 5 per cent. potassium ferricyanide solution. This effects the oxidation to fluorescent thiochrome. About 30 seconds later, 25 ml. of *iso*butyl alcohol (Note 5) are added to extract the thiochrome, and agitation is continued for 60 seconds longer. It is convenient to agitate four tubes at once and oxidise and extract the tubes in pairs, timing being thus simplified. When all the tubes are oxidised, the set is allowed to stand for 10 minutes or longer. During all this work the tubes must be shielded from strong daylight, as thiochrome is photosensitive. (Notes 6 and 7.)

After settling, 1 ml. of ethyl alcohol is added to each tube, and (after all the additions have been made) mixed with the *iso*butyl alcohol layer by bubbling for a second, care being taken that the bubbles do not disturb the surface of the aqueous layer (see Note 8). Each *iso*butyl extract in turn is then used to fill one cell of the Spekker fluorimeter, the other cell being filled with the quinine sulphate solution containing 0·8 µg. per ml. The cells are filled to within about 1/16 inch of the top, the glass lid is placed in position, and all faces are thoroughly wiped. No trace of the bottom aqueous layer in the tubes may be allowed to enter the cell, since it strongly absorbs the active light. It is easier to avoid this if, after the cell has been nearly filled, the tube is restored to the vertical position and rotated on its axis through 90° and the filling of the cell than completed. A background of grey or pale blue blotting paper is a help in the rather dim light that must be maintained.

USE OF SPEKKER FLUORIMETER (see Note 9)—

The quinine cell is placed on the left-hand side of the sliding carrier of the instrument (A, Fig. 1) and need be changed only every second day. The following filters should be placed in the fluorimeter. Before the right-hand photo-cell (B) – Wratten 47 (blue) filter (C) (Note 10). On the right-hand side of the lamp, close to drum support (D), Wood's glass (ultra-violet transmitting) filter (E); on the left, Wood's glass (F) nearest the lamp, then a small circular cell (G) containing the quinine sulphate solution 0·08 g. per 500 ml., followed by neutral glass and coloured or plain glass filters (H, K). The nature and number of these is adjusted in the following manner. With the cell containing the standard quinine solution (0·8 µg. per ml.) in position in front of the right-hand photo-cell (B), the rotating drum (L) of the fluorimeter is adjusted until the fixed arrow points to 0, the light shutter (M) is closed and the zero mark adjusted or marked according to the type of galvanometer and scale, the shutter opened and the iris diaphragm (N) moved to the fully "open" position. The filters are then adjusted until a small movement of the iris diaphragm brings the galvanometer spot back to zero. This will give the maximum sensitivity over the range of normal flour extracts.

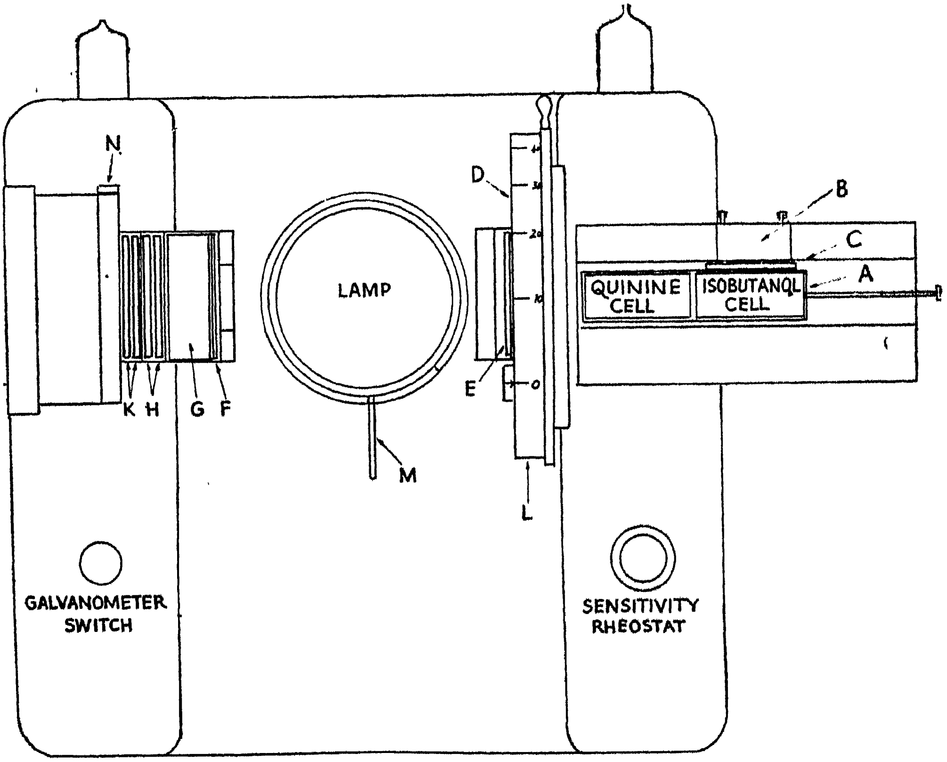

Fig. 1. Sketch Plan of "Spekker" Fluorimeter.

The cell containing the first *iso*butyl extract is gripped by two diagonally opposite vertical edges, using finger and thumb, and placed on the right-hand side of the carrier, which is then moved so that this cell is in the optical beam; spring-loaded stops indicate the correct position (Note 11). The rotating drum of the instrument is set so that the fixed mark is at 0. The light shutter is closed, the galvanometer zero adjusted, the shutter opened and closed twice, with adjustment of the zero if necessary, and the shutter then left open continuously during the examination of the whole run of tubes. The iris diaphragm is now closed until the galvanometer spot is again at zero, and the fluorimeter carrier moved until the quinine cell is in the optical beam. The rotating drum is then moved until once more the galvanometer spot is at zero, whereupon the drum reading is noted and recorded against the standard or extract concerned. Great care must be exercised that no parts are moved at any time during a reading except as mentioned. The remaining tubes are examined in the same manner.

STANDARD CURVES—

A standard curve is prepared by using the whole range of standards from 0·1 to 1·1 µg. per ml., preferably each in duplicate. It will be found that this curve moves slightly from day to day, or even from run to run, so that after a number of standardisations a family of curves (Fig. 2) will be obtained. After this has been done, four standards in each run will suffice to select the appropriate curve for that run. The first, or sometimes the second, tube in each run may be uncertain, owing to the photo-cell taking a little time to reach its correct response under the conditions of working, but this is minimised by the method of zero adjustment described above. It is for this reason that two standards are placed at the end of the set of tubes.

If only one or two samples are to be examined, their extracts may well be included in the run of standards. But at least two runs done at different times will be necessary in this event to justify any confidence in the results.

The curve having been selected in this way, the drum reading of each extract is used to determine the aneurine content in µg. per ml. The curve is conveniently drawn on linear-log paper, with µg. of aneurine on the linear scale, and drum readings on the log scale. Each curve thus obtained is slightly sigmoid, but nearly straight in the middle portion, and the extreme curves of the family are separated by about 12 per cent. of the microgram readings. One or other of these extremes may not be reached for months at a time, but wider departures than mentioned above are usually a sign of dirty electrical contacts or failing photo-cells.

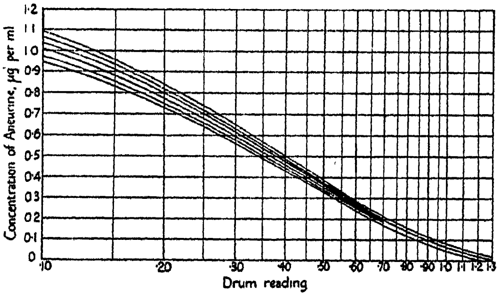

Fig. 2. Standard Curves.

A reproducibility to within ± 0·02 µg. per ml. can be regarded as usual in good work (Note 12).

A blank determination, on 2 ml. of extract, following the same procedure as above, but omitting the potassium ferricyanide, is carried out on all samples until the usual level for the material concerned has been established by many estimations; then it may be found more accurate as well as speedier to take a mean value (*e.g.*, 0·05 µg. per ml. for white flour, 0·02 for wheats). This blank is deducted from the value of the oxidised sample as representing the fluorescence of matter other than thiochrome (see Note 13). It is convenient to determine blanks next to the oxidised sample; they must in any case be determined in the same run. A careful watch on the reagents should be kept by means of blank tubes in which 2 ml. of 0·2 *N* hydrochloric acid are oxidised and extracted in the usual way. Such blanks should be very low (below 1·2 on the Spekker drum).

CALCULATION OF RESULTS—

Results are still officially reported in international units (i.u.) per g., 1 i.u. being equal to 3·125 µg. of crystalline aneurine. Thus:

$$\frac{(\mu\text{g. aneurine/ml.} - \text{blank } \mu\text{g./ml.}) \times \text{total ml. of extract}}{\text{wt. of material taken} \times 3{\cdot}13} = \text{i.u./g.}$$

With the more coloured extracts derived from brans, etc., there is some interference due to absorption of active light in the cells and other causes. This is shown by the fact that somewhat different results are obtained by using extracts of different concentrations, *e.g.*, 2 and 3 g. per 50 ml. The weaker concentration usually gives the higher result. Adsorption methods are available for overcoming this,[6] but a commonly recommended procedure, which is also a valuable check on the accuracy of the whole method, is the addition of pure aneurine to the extracts. This is worse than useless unless carried out with the greatest precision, and the following scheme gives excellent results with homogeneous material. Five portions of material (5 g. of flour or wheat, the latter preferably ground after weighing; 2 g. of bran) are weighed into 100-ml. conical flasks. To each of the first two (labelled "+ 0") are added 50 ml. of 0·2 *N* HCl, to the third (labelled "+ 0·4") 50 ml. of 0·4 µg. per ml. standard aneurine solution, and to the last two (labelled "+ 0·6") 50 ml. of 0·6 µg. per ml. standard, all preferably employing the same 0·2 *N* HCl. Shaking, washing down and standing overnight are carried out as described earlier for the particular material, as is also the subsequent treatment of the extracts. These results give five aneurine concentrations, which when plotted as a graph against aneurine additions (Fig. 3) should give a straight line with divergencies of the order of the experimental error ± 0·02 μg. per ml. in practised work.

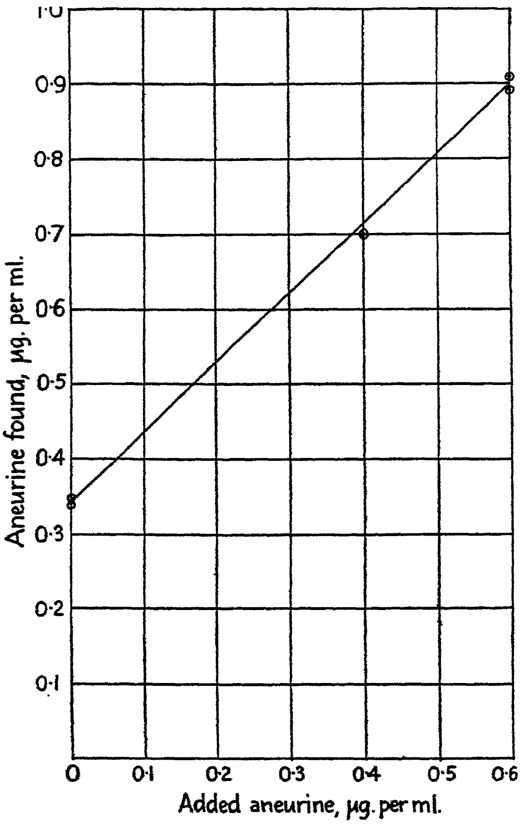

Fig. 3. Recovery of aneurine added to extracts of an 85% extraction flour.

The slope of the line will be nearly 45° with flour—*i.e.*, the "recovery factor":

$$\frac{\text{Aneurine found}}{\text{Aneurine added}} = 98 \text{ to } 102 \text{ per cent.}$$

The recovery is lower with wheats and still lower with brans—possibly as low as 70 per cent. Good recovery is to be regarded as a criterion of reliable results and "corrections" for low recovery must be regarded with suspicion. The causes of low recovery may be complex and this subject will be treated in greater detail in a later publication (Note 14).

NOTES

Note 1—Distilled water is invariably used for washing and steam distillation, as serious trouble was experienced on one occasion from the accumulation of volatile material in the *iso*butyl alcohol, which rapidly reduced ferricyanide and gave erratic results. This appeared to be derived from chlorine in town water and was possibly chloramine. Other volatile fluorescent substances have been found to accumulate in operating on scutellum-rich materials if the first fraction to distil is not returned to residues.

Note 2—This procedure was adopted as giving highly reproducible results. Grinding before weighing, particularly grinding in a disintegrator, was found to result in segregation, which subsequent mixing could not overcome. Weights less than 20 g. may be used, with resulting decrease in accuracy owing to losses in the mill, etc. With cereal products segregation is a very serious problem; errors as great as 20 per cent. can easily arise with coarse wholemeals and similar mixtures. Variation in moisture content within the sample has also been a cause of erratic results.

Note 3—Crystalline aneurine hydrochloride contains approximately one molecule of water of crystallisation. Samples of the National Standard with memorandum may be obtained from the Medical Research Council, Department of Biological Standards, National Institute for Medical Research, Hampstead, London, N.W.3. Larger supplies should be obtained from other sources for use as laboratory standards. 3·125 μg. of the National Standard is stated to be equivalent to 1 international unit.

Note 4—Standards and extracts made up with 0·1 *N* hydrochloric acid have been found to deteriorate with keeping, owing to the growth of organisms. This has not occurred with acid more concentrated than 0·2 *N*. It has frequently been stated that strong solutions of various vitamins keep better than those that are very dilute. This would be contrary to mass action considerations in most cases, and in fact the writer has found with riboflavine as well as aneurine that very dilute solutions keep better than strong if protected from the growth of organisms and, in the case of riboflavine, from light (*cf.* Memorandum on National Standard, see Note 3).

Note 5—Any burette tap or other apparatus used in the estimation must be perfectly free from grease or any material, such as cork or rubber, that can yield fluorescent substances to extracts at any stage.

Note 6—The effect of the variation of every detail has been studied independently of other workers' findings. The accumulated data may be published separately.

Note 7—Special rapid pipettes greatly increase speed, accuracy and safety with this method when many determinations have to be made. An account of these is being published (see this vol, p. 24).

Note 8—The *iso*butyl alcohol extracts of thiochrome may stand for an hour or more without noticeable change if covered by a dark cloth to exclude light. After the addition of the ethyl alcohol they should be examined on the fluorimeter at once, as they become cloudy again on standing. A slight turbidity in the *iso*butanol extract does not influence the result. Hence the liquids in the tubes, after settling at the end of the run, may be poured back into the fluorimeter cell for a check on any particular reading, and this will be found to have changed only slightly to give a higher drum reading.

Note 9—(The adjustments described below should only be undertaken by highly skilled workers.)

Various fittings of the Spekker fluorimeter as delivered were found to cause slight variations in results in such highly sensitive work as aneurine estimation. For this reason the whole of the internal wiring, plug connections, sensitivity resistance and galvanometer switch were removed and replaced by metal-sheathed insulated conductors throughout, joined by the minimum number of carefully soldered and insulated joints, and the outer sheaths bonded. The galvanometer switch, etc. were omitted. The instrument is kept in a warm room, as it was found that some troubles disappeared under these conditions; apparently they were due to traces of condensation in the photo-cell circuit when a humid period followed a cold one. The photo-cell contacts may need careful cleaning when first used, or after standing for some time without use. The mercury lamp is run all day when determinations are being made, the galvanometer lamp being switched on only as required. A constant voltage transformer in the lamp circuit improved steadiness and reproducibility. Needless to say, if an instrument is used in which these alterations have not been made, the sensitivity has to be at a maximum for this work and the galvanometer switch pressed for each galvanometer reading.

Note 10—The Wratten No. 47 filter has been found to be the best commercial filter so far tried for thiochrome. No. 39 was used for a considerable time but in one instance was found to absorb less well a small fluorescence not due to thiochrome.

Note 11—The optical beam should be exactly central in the cell and not touching the glass at any point. If it is not the makers should be consulted.

Note 12—Failure to obtain reproducible results on the *same extract* indicates failure in apparatus or procedure, the most usual being the failure to agitate briskly when adding alkali, or delay after this in adding ferricyanide, which gives low and irregular results. Failure to obtain reproducibility between different extracts may be due to the above causes or to sampling errors, the estimation of aneurine being a severe test of mixing. It was found impossible to mix ground (particularly disintegrator-ground) wheat with sufficient accuracy for the most refined comparison, hence the procedure recommended above. A proportion of commercial flours, particular fortified flours, cannot be mixed to give uniformity. An examination of routine results on National Wheatmeals at one time showed the following divergencies between duplicates (mostly done by different workers in different runs).

Divergency	Proportion of samples per cent.
Less than ± 0·02 i.u. per g.	69
" " 0·03 "	85
" " 0·04 "	93

Most of those showing greater divergency than this failed to give better results on repeated examination.

Note 13—It can be shown that with wheat products the greater part of this blank disappears on oxidation.[6]

Note 14—It should be noted, that for bread, prepared foods and some cereals, digestion[2,3,7] is essential; the oxidation procedure described above can then serve only for examination of extracts prepared less simply than has been described, the accuracy of the whole estimation being lower. Methods recently developed appear likely to give accurate results with these materials.

SUMMARY

The estimation of aneurine (vitamin B_1) in wheat products by the thiochrome method has been used extensively with a steadily increasing refinement and speed. The fullest details of present procedure are described in the attempt to give a greater certainty and ease of approach to those less fully occupied in this field of work.

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RESEARCH ASSOCIATION OF BRITISH FLOUR-MILLERS
CEREALS RESEARCH STATION
ST. ALBANS

January, 1948

High Accuracy and High Speed Pipettes and Associated Apparatus

BY H. N. RIDYARD

A PIPETTE which was designed originally for the accurate measurement of such solutions as bromine in benzene, by combining the principles of the Jakowkin pipette and the pyknometer,[1] was modified shortly afterwards to give still higher accuracy with solutions containing a very volatile constituent that is to be kept constant in concentration (chlorine water originally). More recently the principles employed have been applied to the design of high speed pipettes with an accuracy suitable for many measurements, especially when frequently repeated. Safety with corrosive reagents is another feature of these instruments.

TYPE II, HIGH-ACCURACY PIPETTE FOR VOLATILE SUBSTANCES

The original form of this pipette (Type I), described in 1928,[1] has been modified by dividing the "waste" bulb into two parts by a septum A, having a hole, B, in the upper part. A small bulb, D, above the setting capillary, E, facilitates the setting of the liquid volume before running out the pipette. After the pipette has been filled (conveniently, as originally recommended, by suction at a piece of cycle valve rubber tubing), it is brought into a horizontal position to allow the surplus liquid to fall into the second half of the "waste" bulb, into which is sealed a small air jet, C. After setting the level of the liquid to any point in the narrowest part of the capillary E (a definite mark is unnecessary) by means of a filter paper applied to the tip of the pipette, the liquid can be run out, the incoming air bubbling through the waste liquid at C and so becoming pre-saturated with vapour and in equilibrium with the pipette contents. This device enables extremely volatile substances to be measured.

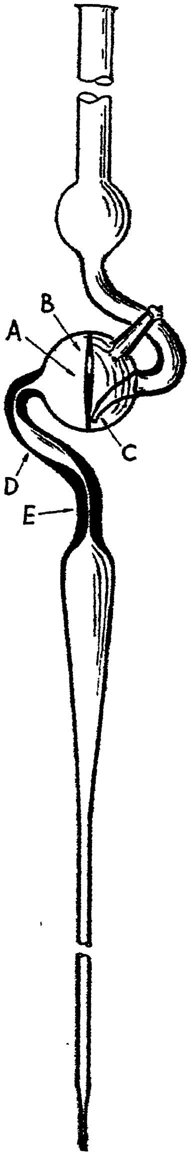

Fig. 1. High accuracy pipette, Type II.

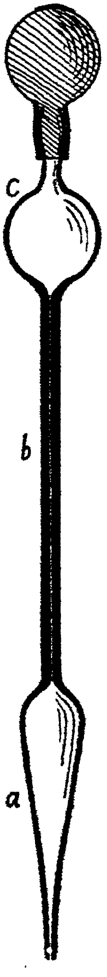

Fig. 2. High speed pipette, Type III.

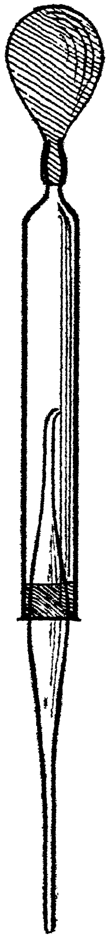

Fig. 3. High speed pipette, Type IV.

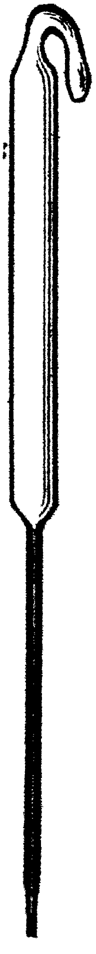

Fig. 5. Inoculation pipette.

The weight of liquid delivered, as stated in the description of the first type, is constant to 1 part in 5000 for water and 1 part in 3000 for benzene for a 2 ml. pipette.

This pipette may have the jet ground to fit a small socket connected to a reservoir so that filling exposes to the atmosphere only that portion of liquid which is taken into the waste bulb.

High speed pipettes

A demand for great numbers of determinations of aneurine (vitamin B_1) in wheat products, each of which requires six separate volume measurements, led to the development of the rapid pipettes (types III, IV and V) described below. These were so successful that they reduced the time of oxidation of a run of 20 tubes from about 35 to 15 mins., and enabled two workers to oxidise the contents of 10,000 tubes per annum.

TYPE III, HIGH-SPEED PIPETTE—

The pipette (Fig. 2) consists of a bulb (*a*) drawn in a uniform taper to a jet at one end, and narrowing sharply from the widest point to a fine capillary (*b*), 3 to 5 inches long, with a bulb (*c*) above, ending in a tubulure to fit a rubber teat or small bulb. As with the earlier pipettes it is most important in the smaller sizes that the capillary and bulb should be drawn from one wide tube, for even the most perfect joint leaves undulations that impede drainage. Pipettes of 0·5-ml. capacity or less may be blown from capillary tube and drawn to shape; in those of 5 ml. or over the error caused by a good glass joint is relatively insignificant. The size of the capillary is of great importance in relation to the liquid for which it is designed, being greater for liquids of higher viscosity. Final adjustment of capacity can if necessary be made by shortening the jet. The pipette is filled by pressing to the required degree on the bulb, allowing to fill until the liquid reaches the fine capillary, whereupon the speed of filling is greatly reduced, giving time for the pipette to be removed from the liquid and its contents ejected into another vessel. It is immaterial to what particular point in the capillary the liquid rises. A practised worker can make 15 measurements per minute. The accuracy of this is shown by the following measurements, which were made by pipetting successive portions of water, etc. into a series of weighing bottles. (All figures are in grams, uncorrected for temperature.)

PIPETTE 1

DESIGNED FOR USE WITH METHYL ALCOHOL (FINE CAPILLARY). NOMINAL CONTENT 2 ML.

	Weights delivered g.		Deviation from mean g.
	2·0293		+ 0·0006
	2·0302		+ 0·0015
	2·0306		+ 0·0019
	2·0214		− 0·0073
	2·0325		+ 0·0038
	2·0280		− 0·0007
	2·0360		+ 0·0073
	2·0260		− 0·0027
	2·0314		+ 0·0027
	2·0212		− 0·0075
	2·0300		+ 0·0013
	2·0278		− 0·0009
Mean	2·0287	Mean	0·0031
		Max.	0·0075

PIPETTE 2

DESIGNED FOR USE WITH CHLOROFORM

Weight delivered Water	Deviation	Weight delivered Chloroform	Deviation
0·495	− 0·019	0·729	− 0·026
0·510	− 0·004	0·746	− 0·009
0·512	− 0·002	0·753	− 0·002
0·520	+ 0·006	0·748	− 0·007
0·519	+ 0·005	0·749	− 0·006
0·513	− 0·001	0·753	− 0·002
0·515	+ 0·001	0·756	+ 0·001
Mean, excluding 1st result:		0·762	+ 0·007
0·514	0·003	0·761	+ 0·006
Max. excluding 1st result:			
0·520	0·006		
		1 hr. later	
		0·754	− 0·001
		0·759	+ 0·004
		0·756	+ 0·001
		0·759	+ 0·004
		0·752	− 0·003
		0·758	+ 0·003
		Mean, excluding 1st and 2nd results:	
		0·755	0·004
		Max., excluding 1st and 2nd results:	
		0·762	0·007

A third pipette designed to deliver 5 ml. of a bacteriological medium gave with nine successive deliveries the following deviations:

	Delivery g.	Mean deviation	Max. deviation
Water: 1st day ..	4·908	± 0·005	− 0·015
2nd day ..	4·921	± 0·011	+ 0·039
Water + glycerol ..	5·364	± 0·028	± 0·087

With this pipette 36 tubes were charged in 3½ minutes.

TYPE IV, HIGH-SPEED PIPETTE—

The above type of pipette proved unsuitable for flour extracts owing to the froth formed when suction is applied, and another type was developed (Fig. 3) in which the first portion entering the pipette is rejected, this serving to remove froth and to wash out the pipette with changing material. This is capable of considerable accuracy with careful use, with a mean deviation of 0·002 on a 2·0 ml. pipette, and a maximum of 0·004, but has not been completely reliable for rapid working. It is hoped to overcome this with modifications which may also improve both speed and accuracy in other types.

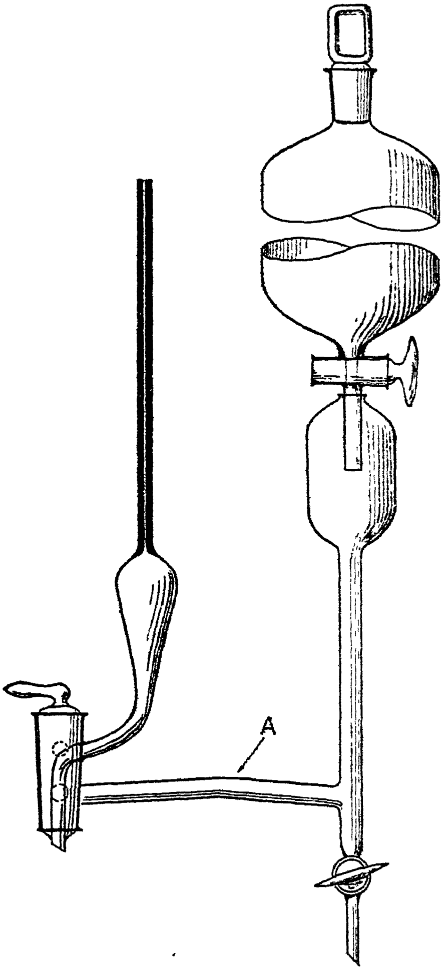

Fig. 4. Rapid reservoir pipette, Type V.

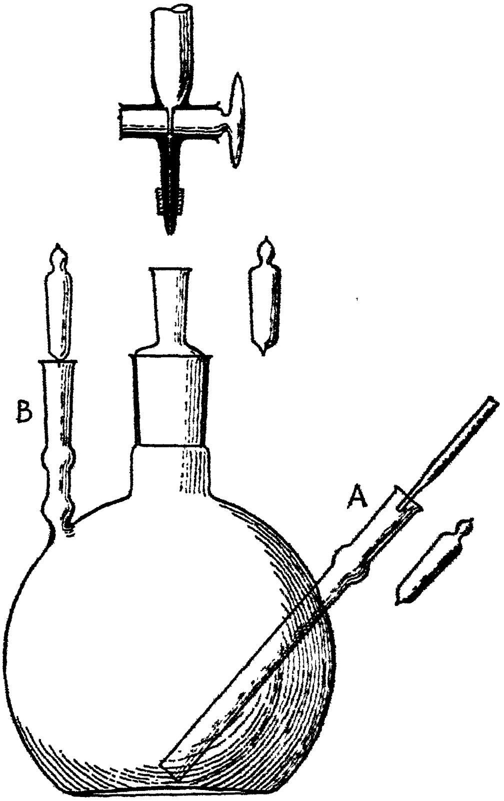

Fig. 6. Titration flask.

(In view of the development of these pipettes originally partly from the pyknometer, it is interesting to notice the considerable degree of similarity of this form with the pyknometer of Cripps,[2] which only recently came to the notice of the present writer.)

TYPE V, RAPID RESERVOIR PIPETTE—

The same form of bulb was used in a reservoir pipette for delivering larger volumes, and this has been made in two slightly different forms. The pipette is shown in Fig. 4. The hollow bore stopcock is so designed that when turned to the "off" position the bulb is filled from the reservoir, *via* two holes drilled in the outer shell of the key; when the stopcock is turned to the "on" position the reservoir is cut off and the contents of the pipette drain out through the inner tube of the key. In the most accurate form the tap is connected to a constant level device as illustrated, so that the liquid rises to the same point in the capillary each time. If the pipette bulb is large, or a lower accuracy sufficient, the pipette may be joined directly to a reservoir at the point A, and then the level of liquid in the capillary will vary with that in the reservoir. Delivery time with this pipette is 20 secs. if it is allowed to drain completely, or 15 if the tap is closed after the continuous stream has ceased and four drops have fallen. With a certain pipette, by the first method, the delivery of water-saturated *iso*butanol (mean of six measurements) was 20·53 g. with a maximum deviation of 0·07 and a mean deviation of 0·036; by the second method 20·54 g. with a maximum deviation of 0·11 and a mean deviation of 0·04. The determinations were made on different days and with different alcohol.

INOCULATION PIPETTE—

Another pipette (Fig. 5) was designed for, and proved of great value in, the inoculation of tubes for microbiological estimations. This consisted of a bulb about 12 cm. long and of 1-cm. bore, drawn out at the top and bent downwards. To the lower end is sealed a length of 0·5 to 1 mm. capillary drawn to a fine jet at the lower end and about 12 to 14 cm. in over-all length. The tubulure at the upper end is packed with a small plug of cotton wool. The whole may conveniently be placed in a glass tube and this plugged with cotton wool and sterilised and left until required. When needed for use the upper end is withdrawn from the sterilising tube and a short length of rubber tube fitted with a screw clip pressed over. The pipette is now completely withdrawn and filled with the saline suspension of the bacteria. It is mounted in a burette stand, and the screw clip adjusted so that drops fall from the lower end of the pipette at such a rate that an operator can take a tube of medium from a rack on his left hand, remove the plug, catch a drop of inoculum in the tube, heat the mouth of the tube, replace the plug, fire and place the tube in a second cage in the correct order. A gross of tubes could be inoculated in about 30 minutes.

TITRATION FLASKS

In order fully to utilise for the titration of volatile materials the high accuracy of the original pipette (Type I), or the first one mentioned in this paper (Type II), a special titration flask was employed.

This had the form shown in Fig. 6. The flask had a neck about 25 mm. in diameter, fitted with a carefully ground stopper, itself carrying a ground neck, of about 7-mm. internal diameter at the top, fitted with a stopper. The flask itself had a stoppered 7-mm. bore tube A sealed through one side and reaching to the bottom, and also carried a small stoppered absorption tube, B. Its use for the estimation of bromine dissolved in volatile solvents will serve as a sufficiently typical example. A few ml. of strong potassium iodide solution are placed in the flask, and a few crystals of solid potassium iodide in the absorption tube, moistened with water. The flask is stoppered, and the small socket of the stopper pressed over a small piece of rubber tube on the jet of the burette to be used. The leg of the pipette containing the liquid to be examined is slipped into the side tube of the flask and the liquid run out beneath the potassium iodide solution, no vapour being lost. The pipette is withdrawn, the side tube stoppered, and the flask gently agitated to aid partition of the bromine. The thiosulphate may now be run in gradually from the burette with agitation, until nearly all the free iodine is removed. The flask is removed from the burette, the contents of the absorption tube are washed into the flask, a few drops of starch solution added, all stoppers are replaced and the flask is vigorously shaken. The last few drops of thiosulphate are added, with vigorous shaking, in this way. Highly reproducible results are obtained.

The high-accuracy pipette type II and the titration flask were designed and employed in the Chemical Department, University of London, King's College, and the author is grateful to Professor A. J. Allmand, D.Sc., F.R.S., for agreeing to their inclusion in this publication.

SUMMARY

Pipettes are described which were designed for high speeds or for measuring volatile materials with high accuracy. A titration flask for use with the latter, and an inoculation pipette are also described.

REFERENCES

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The Assay of Folic Acid with *Streptococcus faecalis* NCTC 6459

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VARIOUS names have been applied to substances having folic acid potency, such as factor SLR, *L. casei* factor and vitamin B_c. These factors have at various times been identified with pteroylglutamic acid, although Hall[1] has recently queried this assumption. They all appear to be available to micro-organisms without preliminary treatment.

The vitamin B_c conjugate, or according to Pfiffner,[2] pteroyl heptaglutamic acid, appears to be split into free, or available, folic acid and an inactive fraction, by the action of hog kidney enzyme. Results have been obtained by Other workers who have used this enzymic treatment for microbiological assay, and have obtained results that compare favourably with bio-assay methods (Bird, Bressler, Brown, Campbell and Emmett).[3]

The folic acid available to the test organism (*S. faecalis*) without preliminary digestion with the conjugase (to liberate folic acid from the conjugated material) has been called "available folic acid" in these experiments.

The present paper deals with a study of the assay of folic acid, available and in the conjugate form. The folic acid used as standard throughout the experiments was supplied by Ashe Laboratories in ampoules containing 2 μg. per ampoule.

The preliminary stages of this work, including all the experiments on the media for growth and subculturing of *S. faecalis*, were carried out in conjunction with Miss M. Meiklejohn, of the Ashe Laboratories.

EXPERIMENTAL

1. MEDIUM USED FOR ASSAY OF FOLIC ACID—

Streptococcus faecalis (*S. lactis R*) was used in the assay of folic acid in preference to *L. casei* for several reasons—

(1) The production of acid by *S. faecalis* is sufficiently rapid to allow of the titration after 16 to 18 hours' incubation, although Tepley and Elvehjem[4] used a 30- to 72-hour incubation period. With an 18-hour incubation period, titration values of 8 ml. of 0·1 *N* sodium hydroxide were found at a 10-mμg. folic acid level, whereas at 72 hours a value of 12 ml. of 0·1 *N* sodium hydroxide was recorded. The assay values obtained with both incubation periods, however, using the same samples, were in all cases in complete agreement, within the limits of the experiment.

(2) The growth of *S. faecalis* may be determined either by titration or by turbidimetric measurements.

(3) *L. casei* is known to be a rather unsatisfactory organism with which to work.

The assay method used was essentially that of Tepley and Elvehjem[4] with very slight modifications. The composition of the basal medium is shown in Table I.

Preparation of the peptone—50 g. of peptone (Difco Bactopeptone) are dissolved in 200 ml. of water, the *p*H is adjusted to 3·0 with concentrated hydrochloric acid and the liquid stirred with 5·0 g. of activated charcoal for 1 hour. After filtration, the volume is adjusted to 500 ml. This solution is stored in the refrigerator under toluene and *renewed at fortnightly intervals.*

Salts B are as for liver tryptone agar.

2. MEDIA USED FOR GROWTH AND SUB-CULTURING OF *S. faecalis*—

Using a 16- to 18-hours incubation period for the assay, the method of maintenance of the stock culture of *S. faecalis* was found to be of critical importance. When the stock culture was maintained in stab culture on Difco yeast extract (or bakers' yeast extract) – sodium acetate – glucose agar and sub-cultured weekly, the response was good for the first four to six weeks. With further sub-culturing, the organism showed little or no response to folic acid.

Although these later sub-cultures of the organism failed to respond to the standard folic acid solution, good growth could always be obtained in an assay containing treated yeast samples. It would appear that the treated yeast samples contained a stimulatory substance or substances required during the first 16- to 18-hours growth, as it was always possible to obtain a good response to the standard folic acid solution when a longer incubation period, 72 hours, was used for the assay.

TABLE I

COMPOSITION OF THE BASAL MEDIUM FOR *S. faecalis*

Casein hydrolysate	5·0 g.
Glucose	20·0 „
Sodium citrate (dihydrate)	25·0
Dipotassium hydrogen phosphate	2·5
L-cystine	0·2
DL-tryptophan	0·4
Asparagine	0·1
DL-alanine	0·2
Adenine	0·01
Guanine	0·01
Uracil	0·01
Xanthine	0·01
Biotin	0·4 µg
Aneurine hydrochloride	200 „
Riboflavine	200
Nicotinic acid	600
Pyridoxine	1200
Calcium pantothenate	400
p-Aminobenzoic acid	10
Peptone treated with charcoal	2·0 ml.
Inorganic salts B	5·0 „
Glass-distilled water to	500

The organism was sub-cultured weekly on the bakers' yeast medium during a period of six months, and tested at intervals. Although the majority of the sub-cultures showed no response to folic acid with the 16 hours incubation period, a good response was occasionally obtained similar to that found with the earliest sub-cultures. No satisfactory explanation has been found for this inconsistent behaviour.

In view of the results obtained by Nymon and Gortner[5] with stock cultures of *L. casei* and *L. arabinosus*, it was decided to alter the medium used for making the sub-culture for *S. faecalis*. The culture was transferred from the bakers' yeast extract agar to liver tryptone broth and from this broth to liver tryptone agar slopes. After the first sub-culture through these two media, the response of the organism to folic acid was regained.

A culture was also transferred from the bakers' yeast extract agar to a takadiastase-treated brewers' yeast agar. In this case, also, the response of the organism was regained in a single sub-culture. Further sub-culturing through liver tryptone broth and agar, or through takadiastase-treated brewers' yeast agar over a period of nine months continued to give satisfactory results.

The results obtained with these sub-culturing media are shown in Fig. 1.

The composition of the media are—

(1) *Liver tryptone broth*—Tryptone (Difco) 1·0 g., K_2HPO_4 0·5 g., glucose 0·2 g., yeast extract (Difco) 0·2 g., liver extract 10 ml. and glass-distilled water to 100 ml.

(2) *Liver tryptone agar*—Tryptone (Difco) 1·0 g., glucose 1·0, K_2HPO_4 0·2 g., $CaCO_3$ 0·3 g., agar 2·0 g., liver extract 10 ml., salts A 0·5 ml., salts B 0·5 ml. and glass-distilled water to 100 ml.

The liver extract is prepared by suspending one pound of ground fresh liver in 2 litres of water, heating for 60 minutes on a steam bath and then filtering through cheese cloth. The filtrate is neutralised to *p*H 7·0, heated again for 15 minutes, filtered through coarse filter paper and stored under toluene in a dark bottle in the refrigerator.

(3) *Salts A*—K_2HPO_4 25 g., KH_2PO_4 25 g. and glass-distilled water to 250 ml.

(4) *Salts B*—$MgSO_4.7H_2O$ 10·0 g., $MnSO_4.4H_2O$ 0·5 g., $FeCl_3.6H_2O$ 0·03 g., concentrated HCl 5 drops and glass-distilled water to make 250 ml.

(5) *Takadiastase-treated brewers' yeast medium*—Pressed brewers' yeast (25 per cent. solids) 50 g., 1 per cent. sodium acetate buffer solution of *p*H 4·5 500 ml., takadiastase 1·0 g.

The mixture is incubated for 18 hours at 37° C., neutralised to *p*H 7·0 and finally autoclaved for 15 minutes at 15 lb. pressure. After filtration the volume of the solution is adjusted to 500 ml. and the following are added, glucose 2 g., salts B 5 ml. and agar 10 g. The whole is steamed to dissolve the agar, and finally autoclaved 15 minutes at 15 lb. pressure after distribution in the tubes.

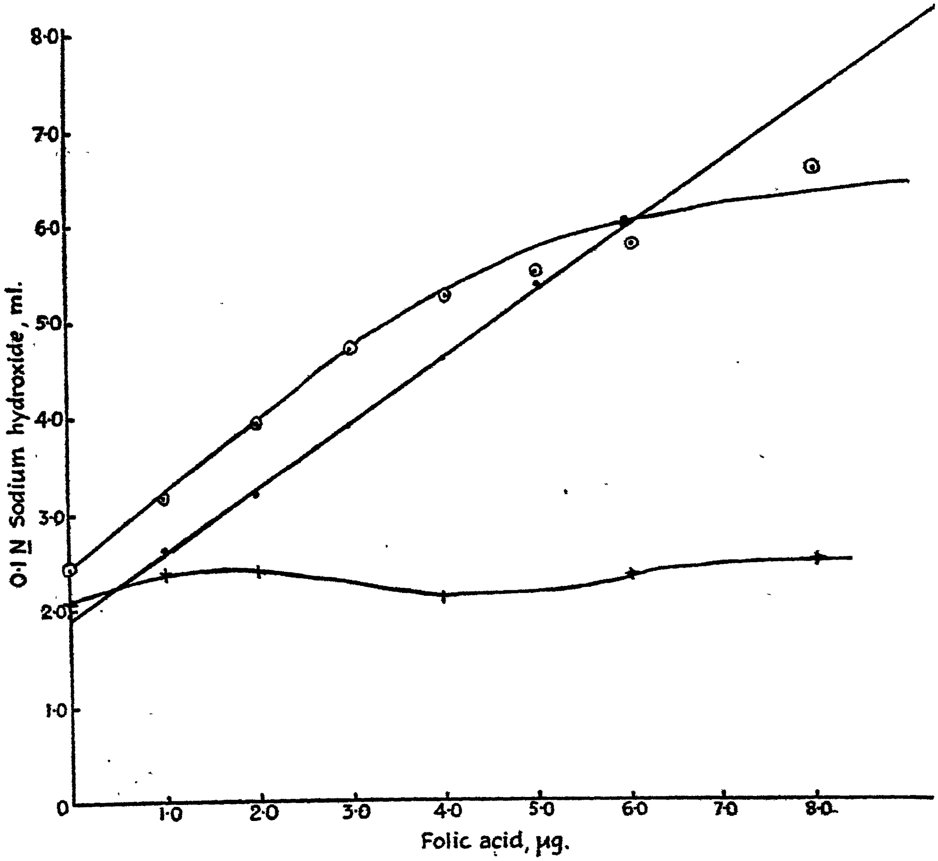

Fig. 1. The effect of the sub-culturing medium on the efficiency of *S. faecalis* for folic acid assay.

⊙ Bakers' yeast medium—1st sub-culturing.
+ Bakers' yeast medium—6th sub-culturing.
● Liver tryptone broth and agar.

3. EFFECT OF TAKADIASTASE IN THE PREPARATION OF SAMPLES FOR ASSAY—

When assaying yeast samples for available folic acid content, using taka diastase according to the method of Cheldelin, Eppright, Snell and Guirard,[6] very irregular results were obtained. It was therefore decided to investigate the effect of takadiastase on the standard folic acid solution.

To 0·2 μg. of folic acid in 8·0 ml. of an acetate buffer solution, *p*H 4·5 to 4·6, were added 0·02 g. of takadiastase and a few drops of toluene. The solution was incubated at 37° C. for 16 to 18 hours. As a control, 0·2 μg. of folic acid in the same buffer solution was treated in the same manner, but without the enzyme. After adjusting the *p*H to 7·0 the solutions were steamed for 15 minutes to inactivate the enzyme and finally cooled and diluted for assay.

The results are shown in Table II.

TABLE II

EFFECT OF TREATING STANDARD FOLIC ACID SOLUTION WITH TAKADIASTASE

	Folic acid added mμg.	Folic acid found mμg.	Found %
Control	2·0	1·8	90
	4·0	4·2	105
Takadiastase-treated standard solution.	2·0	0·5	25
	4·0	1·3 3·0	33 75
	6·0	2·6 4·2	43 70
	8·0	4·5 5·6	56 70
	10·0	6·9	69

These results show that there may be a loss of 25 to 75 per cent. of folic acid on treatment with takadiatase, whilst the incubated control shows little or no loss.

In order to show whether this effect was due to the action of the inactivated enzyme on the folic acid during the incubation period of the test, 0·02 g. of takadiastase was incubated in the acetate buffer solution, neutralised, steamed and diluted to one quarter of the previous volume. Two ml. of this solution were added to a series of tubes containing folic acid, and these assayed for folic acid content in the usual manner. From Table III it is clear that the inactivated enzyme, when added at four times the normal strength to folic acid dilutions in the test itself, does not lead to any loss of folic acid activity.

TABLE III

EFFECT OF ADDING A CONSTANT AMOUNT OF INACTIVATED TAKADIASTASE TO FOLIC ACID IN THE TEST

Folic acid added, mμg.	0	1·0	2·0	3·0	4·0	5·0	6·0
Folic acid found, mμg.	0	1·1	2·0	3·1	4·2	5·3	6·0

A sample of dried yeast was then incubated in the acetate buffer with takadiastase, and also with addition of 0·2 μg. of folic acid. These samples were neutralised, steamed and diluted for assay. The results are shown in Table IV.

TABLE IV

EFFECT OF TREATING A YEAST SAMPLE WITH TAKADIASTASE

Dried yeast 1 : 1000	Test solution taken, ml.	Folic acid added, mμg.	Folic acid found, mμg.	Folic acid, μg. per g. of yeast	Maximum % found
Yeast alone	1·0	0·0	0·8	0·8	
	2·0	0·0	1·4	0·7	
	3·0	0·0	2·0	0·67	
	4·0	0·0	2·5	0·63	
Yeast + Folic acid.	0·5	1·0	0·9		66
	1·0	2·0	1·5		55
	2·0	4·0	3·5		65
	3·0	6·0	5·5		68

From all the results, both with the folic acid and with dried yeast samples, it is clear that the use of takadiastase is unsatisfactory in the preparation of samples for assay purposes. The noticeable "drift" in the results for yeast alone would indicate that the takadiastase is interfering with the assay.

4. EFFECT OF 1 PER CENT. SODIUM ACETATE SOLUTION AND OF 0·1 *N* HYDROCHLORIC ACID IN THE PREPARATION OF SAMPLES FOR ASSAY—

In view of the unsatisfactory results obtained with takadiastase to extract the available folic acid, yeast samples were prepared for assay by autoclaving at 15 lb. pressure from

15 to 60 minutes either in 1 per cent. sodium acetate solution, *p*H 4·0 to 4·2, or 0·1 *N* hydrochloric acid or by steaming for 15 minutes in 0·1 *N* hydrochloric acid. Values obtained after treatment of samples by these methods are shown in Table V.

TABLE V

VALUES OBTAINED FOR AVAILABLE FOLIC ACID CONTENT OF A DRIED YEAST SAMPLE TREATED WITH 1 PER CENT. SODIUM ACETATE SOLUTION AND WITH 0·1 *N* HCl

Treatment of yeast	Time of autoclaving, mins.	Folic acid, µg. per g.
Sodium acetate ..	15	1·18
	30	1·51
	45	1·81
	60	2·21
0·1 *N* HCl ..	15	2·0
	30	2·26
	45	2·25
	60	2·05
0·1 *N* HCl steamed ..	15	0·83

From these experiments, it can be seen that the treatment with 0·1 *N* hydrochloric acid gives higher values for available folic acid from the dried yeast. When folic acid itself was treated in the same manner, there was a considerable loss, a recovery of 35 to 45 per cent. being recorded.

TABLE VI

EFFECT OF PRIOR TREATMENT WITH TAKADIASTASE, 0·1 *N* HCl AND SODIUM ACETATE ON THE AVAILABLE FOLIC ACID CONTENT OF VARIOUS FOODSTUFFS

	Folic acid content in µg. per g. of sample treated with		
Food	Takadiastase	0·1 *N* HCl by autoclaving for 15 min. at 15 lb. pressure	Sodium acetate by autoclaving for 15 min. at 15 lb. pressure
Dried yeast	0·86	3·95	1·25
Dried yeast tablets	0·73	1·00	0·77
Sacc. cereviseae with bacteria (acetone dried sample).	2·00	2·80	2·60
Dried whole milk	nil	nil	nil

On the other hand, the yeast sample, when treated with acetate buffer, showed with increasing time of autoclaving an increase in the values for available folic acid, which may indicate a gradual release of the conjugated material to a form available to the test organism. Folic acid treated in a similar manner gave a recovery of 90 to 105 per cent.

The effect of the three different forms of treatment on various foodstuffs is shown in Table VI. From the results, it is obvious that there is no reliable method at present for the assay of available folic acid in foodstuffs such as yeast, in which the folic acid is, in part at least, held inside the cell wall. The agents that can be used to destroy the cell wall and liberate the folic acid appear to breakdown the conjugate. For this reason it has been decided to ignore the available folic acid and assay the total folic acid, available and conjugated. Justification for such a procedure lies in the fact that both forms appear to be equally available and equally utilised in the animal body.

5. EFFECT OF LIGHT ON FOLIC ACID—

The effect of artificial light and of ultra-violet light on folic acid was studied by exposing a solution of folic acid containing 0·2 µg. per ml. to the rays from either a 100-watt lamp for 4 hours, or to ultra-violet light (1849 to 4500 A.) for 2 hours. All solutions were exposed directly in layers 10 mm. in depth in crystallising dishes, at a distance of 30 cm. from the light source and, after exposure for the given time, diluted for assay.

The solutions exposed to the ultra-violet light showed a loss of folic acid of 33 per cent. after 30 minutes, 40 per cent. after 60 minutes, 55 per cent. after 90 minutes, and 72 per cent. after 120 minutes exposure. The solutions exposed to the rays of the 100-watt lamp showed no loss of potency after 4 hours exposure.

Thus ordinary electric light seems to have little or no effect on the folic acid solution containing 0·2 µg. per ml. after a fairly long exposure, but some loss will occur after a comparatively short exposure to ultra-violet light.

6. EXTRACTION OF THE FOLIC ACID FROM THE VITAMIN B_c CONJUGATE—

In order to liberate the folic acid from its conjugated form for assay with *S. faecalis*, the method of Bird *et al.*[3] with slight modifications, was used. Dried hog kidney was prepared by mincing one or more fresh kidneys, adding five volumes of acetone, filtering, washing with acetone and finally air-drying. The dried kidney was ground as finely as possible and stored in the refrigerator.

The weight of dried kidney required to liberate the maximum amount of folic acid from the conjugate in 18 hours at 40° C. was found by taking a constant amount (0·1 g.) of a dried yeast, adding various amounts (0·01 to 0·10 g.) of dried kidney and incubating in 8·0 ml. of 1 per cent. sodium acetate solution, *p*H 4·0 to 4·2, with addition of a few drops of toluene. After incubation, the samples were neutralised to *p*H 7·0, steamed for 15 minutes and, after cooling, diluted for assay. From the results recorded (Table VII) it is clear that 0·03 g. of dried kidney is sufficient to liberate the folic acid from its conjugated form in 18 hours from 0·1 g. of dried yeast. Similar results were obtained with other samples of dried kidney.

TABLE VII

AMOUNTS OF FOLIC ACID LIBERATED FROM A DRIED YEAST BY VARIOUS AMOUNTS OF DRIED KIDNEY IN 18 HOURS AT 40° C.

Dried kidney, g.	µg. Folic acid, per g. of dried yeast
0·01	31·6
0·02	38·5
0·04	35·5
0·06	34·1
0·08	38·4
0·10	39·5

The blank value for 0·03 g. of the dried kidney was comparatively low, and remained constant for the same batch during four months' storage.

The maximum amount of folic acid appeared to be liberated in 18 hours at 40° C. with 0·03 g. of kidney, as no increase in the folic acid content of dried yeast was found with longer incubation periods of 24, 48, or 72 hours. When folic acid alone was treated in the same manner with 0·03 g. of dried kidney for 18 hours at 40° C. a recovery of 95 to 105 per cent. was always obtained.

7. A COMPARISON OF VALUES OBTAINED BY THE USE OF *S. faecalis* AND *L. casei* AS TEST ORGANISM FOR THE ASSAY OF FOLIC ACID—

Although *S. faecalis* is frequently used as test organism for the assay of folic acid, statements have been made that *L. casei* is preferable, yielding more accurate results. *L. casei* is certainly much more sensitive to folic acid, more especially at the lower concentrations. It was felt, however, that this difference in sensitivity of *L. casei* would only become apparent and significant when dealing with trace quantities of folic acid in the test material. In view of the contradictory statements on this subject it was decided to compare the results obtained using the two organisms on the same series of test solutions.

A series of preliminary experiments with *L. casei* showed that it was impossible to use the same assay medium as was used with *S. faecalis*. The failure of this medium was due to the presence in the vitamin-free casein hydrolysate of some substance or substances which could replace folic acid for *L. casei*. That it was not folic acid was seen from the fact that it did not act as a growth factor for *S. faecalis*. In view of this, an amino acid basal medium was prepared, similar to that proposed by McMahan and Snell[7] for use with *L. casei* and *L. arabinosus* in the assay of valine and arginine. A strepogenin concentrate was prepared according to the method of Sprince and Woolley[8] and added to the medium. Choline chloride and inositol were found to be unnecessary and were omitted from the medium.

The organisms for the inoculum were maintained on liver tryptone agar, *L. casei* as a stab culture, *S. faecalis* on slopes. For the inoculum, *L. casei* was grown in 10 ml. of liver tryptone broth for 18 hours at 37° C., washed twice with 0·9 per cent. salt solution, suspended in 20 ml. of salt solution and finally diluted by adding 2 ml. to 50 ml. of salt solution. One drop of this suspension was added to each assay tube.

Adequate washing of the organisms is essential, otherwise a response may possibly be obtained even in absence of any added folic acid. Further, the volume of the inoculum added to the basal medium is very important. When more than one drop of the final diluted suspension of organisms was added, maximum growth and acid production was frequently found when no folic acid was added. It is possible that the extreme sensitivity of *L. casei* for folic acid found by other workers may be due to inadequate washing of the organisms and inadequate care in the addition of the inoculum to the basal medium or to a lack of knowledge of the importance of these factors.

The results of a comparison of *S. faecalis* and *L. casei* for the assay of folic acid are given in Table VIII.

TABLE VIII

COMPARISON OF THE ACTIVITY OF *S. faecalis* AND *L. casei* FOR THE ASSAY OF FOLIC ACID

Material	Folic acid (μg. per g.) as assayed by	
	S. faecalis	*L. casei*
Hog kidney	10·0	10·0
"	8·7	8·4
Dried milk	nil	nil
"	"	"

With the materials used it is clear that both organisms are effective for assay purposes. It is fully realised that there are certain other factors, the deficiency or presence of which may affect the growth of one or other of these organisms, but it would appear that, as regards the assay of folic acid *per se*, either organism may safely be used.

At this stage, a further precaution in the assay of folic acid with *S. faecalis* must be noted.

Test solutions assayed after storage for five days at 4° C. were not infrequently found to give higher values than when assayed immediately after preparation. All solutions for folic acid assay must be tested immediately on preparation and not stored, otherwise unreliable results may be obtained. Obviously some other growth factor capable of replacing folic acid is being formed slowly by the interaction or breakdown of the compounds originally present.

8. RECOMMENDED METHOD OF ASSAY OF FOLIC ACID WITH *S. faecalis*—

(*a*) *Treatment of the sample*—Weigh 0·1 g. of the sample containing 10 to 50 μg. of total folic acid per gram and 0·03 g. of desiccated hog kidney into a 50-ml. conical flask; add 8·0 ml. of acetate buffer (1 per cent. sodium acetate solution, *p*H 4·0 to 4·2) and a few drops of toluene and incubate at 40° C. for 18 hours. After neutralisation to *p*H 7·0 with sodium hydroxide solution, steam the suspension for 15 minutes, cool, dilute to 10 ml. and filter. A suitable dilution of the filtrate, estimated to contain approximately 2 mμg. of folic acid per ml. is taken for assay.

(*b*) *Method of assay*—Into a series of test-tubes measure 5·0 ml. of the basal medium, *p*H 6·8, given in Table I, followed by suitable aliquots of the test material, four levels in triplicate, in quantity such that the amount of folic acid per tube ranges from 1·0 to 8·0 mμg.

Set up in triplicate a series of tubes for the standard, containing 0, 1·0, 2·0, 4·0, 6·0, 8·0 and 10·0 mμg. of folic acid.

After making up the contents of all the tubes, both test and standard, to 10 ml. with water, autoclave at 10 lb. pressure for 10 minutes.

Inoculate each tube with one drop of a twice washed *S. faecalis* culture re-suspended in 20 ml. of 0·9 per cent. salt solution, and then incubate at 30° C. for 18 hours.

Titrate the acid produced with 0·1 *N* sodium hydroxide to *p*H 8·5, adding thymol blue to each tube as indicator.

9. ASSAY OF VARIOUS FOODSTUFFS—

The results of assays of different samples of brewers' yeast, milk and other foods are given in Table IX. It will be noted that the foods, other than yeast, contained only minimal quantities of folic acid.

Samples tested from weekly batches of pressed brewers' yeast, all containing approximately 25 per cent. of total solids, were found to vary considerably in folic acid content. Similar variations from week to week were also found in the pyridoxine content.

TABLE IX

THE TOTAL FOLIC ACID CONTENT OF VARIOUS FOODSTUFFS

	No. of samples	Folic acid, µg. per g.
Dried yeast tablets	4	5·0
Dried yeast (brewers')	10	35·5
Saccharomyces cereviseae acetone-dried (pure culture)	2	20·9
Saccharomyces + bacteria, acetone dried	2	27·5
Pressed yeast (calculated on dry weight)		
(i)	2	21·2
(ii)	2	17·0
(iii)	2	18·8
(iv)	2	7·4
(v)	2	26·5
(vi)	2	23·6
(vii)	2	17·5
Dried full-cream milk	5	Nil to trace
Fresh cows' milk	2	"
Poultry mash	2	0·78
Dairy nuts	2	0·85
Wheat	2	trace
Maize meal	2	"
Oats	2	"

SUMMARY

1. A study has been made of the method of assay of total folic acid using *S. faecalis* NCTC 6459.

2. No method has been found so far, for the assay of free folic acid, owing either to the reaction of added material with the folic acid, as when takadiastase is used, or to the partial splitting of the conjugate, as in autoclaving with 0·1 *N* hydrochloric acid.

3. Ordinary artificial light has little or no effect on folic acid. Some loss occurs after a comparatively short exposure to ultra-violet light.

4. A method is given for the maintenance of the culture of *S. faecalis*, consisting of serial sub-culture through liver tryptone broth and agar.

5. A comparison of assay results using *S. faecalis* and *L. casei* has shown that, with the materials assayed, and when certain precautions are taken, there is little or no difference in the results.

6. Assays of brewers' yeast, dried milk and various foods by the recommended method are included.

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A Study of the Stokes Method for the Assay of Vitamin B_6 with *Neurospora sitophila* M.299

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SEVERAL methods for the assay of vitamin B_6 have been advocated at different times, but only two are in general use, a gravimetric method using the mould *Neurospora sitophila M*.299 and a turbidimetric method using the yeast *Saccharomyces carlsbergensis*. In both, the total vitamin B_6 activity is assayed, whereas, by using *Lactobacillus casei* and *Streptococcus faecalis* (Snell and Rannefeld[1]) the pyridoxal and pyridoxamine fractions respectively are determined. The use of *S. faecalis* is considered extremely unsatisfactory on account of the very variable response of the assay organism.

The turbidimetric method has a short incubation period and there is no necessity to remove aneurine present (Atkin, Schultz, Williams, and Frey[2]). It has, however, the disadvantages of requiring a readily controllable shaker and a sensitive photo-electric colorimeter, which are not available in every laboratory. Further, the degree of accuracy obtainable is by no means high, and the duplication of results not infrequently poor.

The gravimetric method, using *N. sitophila*, has an incubation period of five days and aneurine must be removed. Nevertheless the method has the advantage of being gravimetric and relatively simple.

Various methods of extracting pyridoxine from different materials, in a form readily available to the assay organism, have been suggested. Rubin, Scheiner, and Hirschberg[3] used 0·055 *N* sulphuric acid or treatment of the sample with an enzyme preparation, for extraction of the vitamin B_6. Since *Saccharomyces carlsbergensis* was used as assay organism, it was unnecessary to remove the aneurine present in the extract. Rabinowitz and Snell[4] also used 2 *N* sulphuric acid as the extractant in a series of differential assays with *Sacch. carlsbergensis*, *L. casei*, and *Strept. faecalis*.

With *Neurospora sitophila* as assay organism, acid hydrolysis of the test sample must be followed by further treatment to remove any aneurine present. Stokes[5] has advocated the use of sodium sulphite followed by hydrogen peroxide to destroy the aneurine. This treatment has not, however, given either accurate or reproducible results. A study has, therefore, been made of this method and the modifications necessary to obtain accurate results. The effects of the age of the mould culture, of visible and ultra-violet light and of aneurine on the assay are included, together with the results of a series of assays of different foods.

Throughout, the term vitamin B_6 refers to the complex containing pyridoxine, pyridoxal, and pyridoxamine.

EXPERIMENTAL

1. MEDIUM USED FOR GROWTH AND SUB-CULTURING OF *N. sitophila*—The resting spores of the mould were maintained in a 10 per cent. cane sugar medium and transferred to a solid medium three days or more before a culture was required for assay purposes. This solid medium consisted of maltose 15·20 g., Bactopeptone Eupepton 3·20 g., malt extract (70 per cent. solids) 1·14 g., agar 8·00 g., and water to make up 400 ml.

A slope was incubated for three days at 30° C. and a loopful of the culture added to 70 ml. of sterile water for use as inoculum.

2. MEDIUM USED FOR THE ASSAY OF PYRIDOXINE—The composition of the basal medium used in the assays was based on that advocated by Stokes, Larson, Woodward, and Foster.[6] It consisted of sucrose 7·50 g., ammonium tartrate 2·50 g., potassium dihydrogen phosphate 1·25 g., sodium dihydrogen citrate 1·00 g., inorganic salt solution A 10 ml., inorganic salt solution B 10 ml., biotin 2 μg., and water to make up 250 ml.

Inorganic salt solution A consisted of 2·5 g. of $MgSO_4{\cdot}7H_2O$ and 0·01 g. of $ZnSO_4.7H_2O$ per 100 ml. of water and solution B of 0·5 g. of NaCl, 1 g. of $CaCl_2.6H_2O$ and 0·167 g. of $FeCl_3$ per 100 ml. of water.

3. EFFECT OF THE AGE OF THE CULTURE OF *N. sitophila*—With certain organisms, such as *S. faecalis*, sub-culturing on the same medium for a comparatively short period of time leads to a partial failure of the organism to respond to a vitamin normally essential for

growth. Nymon and Gortner[7] altered the growth medium for *L. casei* and obtained an increased response of the organism in riboflavine assays. A similar change of medium has been found essential with *S. faecalis* in the assay of folic acid (Jones and Morris[8]).

It was, therefore, decided to investigate the length of life of *N. sitophila* for pyridoxine assay. A series of cultures of *N. sitophila* was taken from the original 10 per cent. cane sugar culture to a solid malt extract - peptone medium at intervals of approximately one month. These sub-cultures were stored in the refrigerator until tested. Five such sub-cultures, varying in age from one to five months, were examined and compared with a fresh culture. The results, shown in Table I, show that little or no change in activity takes place as a result of maintaining a culture over a fairly long period (five months) on the malt-extract - peptone medium. The variations between one series of results and another bear little or no relationship to the age of the culture and are the normal daily variations that might be expected with the same culture. For this reason it is essential and customary to arrange a series of control experiments with each series of test solutions.

The results obtained warrant the assumption that, unlike certain bacterial cultures such as *S. faecalis*, the mould *N. sitophila* retains its activity for the assay of pyridoxine over fairly long periods.

TABLE I

EFFECT OF AGE OF CULTURE USED IN ASSAY

Pyridoxine, µg.	Weight of mould mycelium in mg.					
	144 days old	126 days old	98 days old	67 days old	44 days old	12 days old
0·1	6·8	6·6	5·5	6·6	6·2	7·2
0·4	15·7	16·6	16·6	16·9	20·9	17·3
0·8	32·2	34·7	35·8	36·1	36·6	33·1
1·6	37·4	41·8	37·1	46·3	41·2	42·6

4. EFFECT OF ANEURINE ON THE ASSAY OF PYRIDOXINE—Aneurine is known to affect the assay of pyridoxine with *N. sitophila*, but it has never been clearly shown whether aneurine is an essential nutrient for the mould. Thus, Barton-Wright[9] states that in presence of aneurine the mould no longer responds quantitatively to additions of pyridoxine.

A series of experiments was carried out to determine whether aneurine is an essential vitamin for the growth of *N. sitophila*, and whether the presence of aneurine in a pyridoxine solution gives an additive effect.

One ml. of an aqueous solution of pyridoxine containing 0·2 µg. was added to a series of tubes of basal medium and followed by different quantities of an aqueous solution of aneurine containing 0·2 µg. per ml. After diluting each to 10 ml. the tubes and contents were autoclaved at 15 lb. pressure for one hour, cooled and inoculated with *N. sitophila*. They were then incubated for five days and the mycelium weighed. Conjointly, two further tests were carried out, one in which aneurine alone was added to the basal medium, and the other in which pyridoxine alone was added.

Fig. 1 shows that there is a linear relationship between the amount of aneurine present in the medium and the growth response of the mould, as measured by the weight increase. The response with aneurine is, however, relatively small when compared with that found with pyridoxine. Further, there are occasions when the mould shows no response to addition of aneurine.

The results show that the presence of aneurine may interfere markedly with the assay of pyridoxine, possibly in an additive manner. It is obvious, therefore, that aneurine must be removed from any solution which has to be assayed for pyridoxine.

5. EFFECT OF SODIUM SULPHITE AND HYDROGEN PEROXIDE ON PYRIDOXINE—The method advocated for the destruction of aneurine, when present in solution together with pyridoxine, is by addition of sodium sulphite, any excess of sulphite being removed by means of hydrogen peroxide. The *p*H of the sulphite-treated material must be 8, and a slight excess of peroxide is necessary to destroy all the sulphite, otherwise the mould will not grow. It is known, however, that both sulphite and peroxide attack pyridoxine, although Cunningham and Snell[10] found that the loss of pyridoxine was minimal between *p*H 8 and 8·6.

In view of the possible source of error in this method of preparing extracts, a series of experiments was arranged in which solutions of pyridoxine in water were treated with sodium sulphite at *p*H 8, and steamed at 100° C. for 30 minutes, and the sulphite finally removed with hydrogen peroxide. Any excess of peroxide was destroyed when the medium was sterilised. The results, an average of three separate experiments, are shown in Table II.

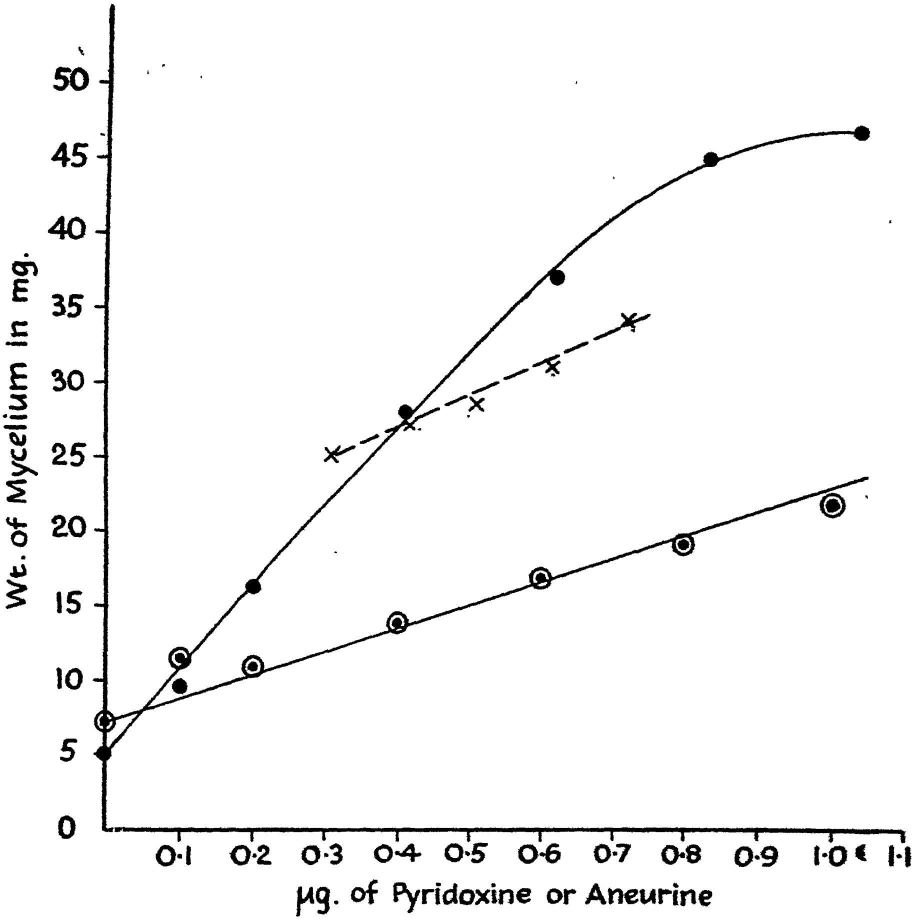

Fig. 1. Curves showing the effect of aneurine alone, pyridoxine alone and mixtures of aneurine and pyridoxine on the growth of *N. sitophila*.

● Pyridoxine alone.
⊙ Aneurine alone.
× Pyridoxine and aneurine mixtures.

It is clear that, even when all precautions are taken, the sulphite - peroxide treatment affects the recovery of pyridoxine from pure solution. The results show an average loss, in the present series of experiments, of about 35 per cent.

TABLE II

EFFECT OF SODIUM SULPHITE AND HYDROGEN PEROXIDE ON THE ASSAY OF PYRIDOXINE

Pyridoxine added, μg.	Pyridoxine found, μg.	Percentage loss
0·1	0·07	30
0·2	0·15	25
0·4	0·28	30
0·6	0·43	28
0·8	0·50	38
1·0	0·59	41

With brewers' yeast as the test material, a series of pyridoxine assays was carried out in a similar manner, and the results (Table III) show a variation of the order of 100 per cent., clearly indicating that little or no reliance can be placed on the method of analysis.

TABLE III

ASSAY OF VITAMIN B_6 CONTENT OF BREWERS' YEAST COMPARING Na_2SO_3 AND NaOH FOR THE INACTIVATION OF ANEURINE

Amount of final diluted sample taken, ml.	Vitamin B_6 found, µg. per g.	
	Na_2SO_3 treatment	
	Yeast 1	Yeast 2
1	8	6
2	9	8
3	10·3	11
4	15	12·4

6. EFFECT OF SODIUM HYDROXIDE ON THE ASSAY OF PYRIDOXINE—In view of the results of the experiments in which sodium sulphite was used to remove the aneurine, this method for the assay of pyridoxine had to be abandoned. Fortunately, however, aneurine is particularly sensitive to the presence of alkali, whereas pyridoxine is stable. It is stated that none of the pyridoxine group of vitamins is affected by heat treatment in presence of *N* sodium hydroxide (Cunningham and Snell[10]).

A series of experiments was arranged to determine whether sodium hydroxide affected pyridoxine, pyridoxal, and aneurine. The pyridoxal used was a sample of the pure product supplied by Merck or a sample made according to the method given by Barton-Wright. In the first series pyridoxine was treated with *N* sodium hydroxide and assayed. Pyridoxal was treated in a similar manner. The results are given in Table IV. To investigate the effect of *N* sodium hydroxide on aneurine, different quantities of a solution of pyridoxine were added to 1 ml. of an aqueous solution containing 1 µg. of aneurine. The solution was autoclaved at 15 lb. pressure for 1 hour with *N* sodium hydroxide and assayed for pyridoxine. The results are given in Table V. Finally, 1 ml. of an aqueous solution of pyridoxine, containing 0·2 µg. was added to different quantities of an aqueous solution of pyridoxal. The mixtures were assayed before and after treatment with sodium hydroxide. The results are given in Table VI. The complete results show that, whereas aneurine is completely inactivated by heating in presence of sodium hydroxide, pyridoxine and pyridoxal are relatively unaffected.

TABLE IV

EFFECT OF *N* NaOH ON THE ASSAY OF PYRIDOXINE AND PYRIDOXAL

Pyridoxine		Pyridoxal	
Amount added, µg.	Amount found, µg.	Amount added, µg.	Amount found, µg.
0·10	0·08	0·18	0·20
0·20	0·21	0·36	0·36
0·40	0·37	0·54	0·55
0·60	0·60	0·72	0·66
0·80	0·84	0·90	0·86
1·00	1·10	—	—

TABLE V

EFFECT OF *N* NaOH ON THE ASSAY OF PYRIDOXINE IN THE PRESENCE OF ANEURINE

Pyridoxine added, µg.	Aneurine added, µg.	Pyridoxine found, µg.
0·10	1·00	0·08
0·20	1·00	0·22
0·30	1·00	0·33
0·40	1·00	0·41
0·50	1·00	0·54

TABLE VI

EFFECT OF *N* NaOH ON THE ASSAY OF MIXTURES OF PYRIDOXINE AND PYRIDOXAL

Pyridoxine added, µg.	Pyridoxal added, µg.	Total found, untreated, µg.	Total found treated with *N* NaOH, µg.
0·20	0·05	0·27	—
0·20	0·23	0·50	0·42
0·20	0·50	0·67	0·69
0·20	0·68	0·75	0·80

7. EFFECT OF DIFFERENT QUANTITIES OF SODIUM HYDROXIDE AND OF SODIUM HYDROXIDE FOLLOWED BY ACID ON THE EXTRACTION OF VITAMIN B_6 FROM YEAST—Rubin, Scheiner, and Hirschberg found that extraction with 0·055 *N* sulphuric or hydrochloric acid was essential to free all the B_6 vitamins. Following hydrolysis of the test sample with 0·1 *N* sodium hydroxide, in which only a small fraction of the B_6 group was extracted, a further treatment with 0·055 *N* sulphuric acid extracted all the vitamin B_6. For assay purposes they used *Saccharomyces carlsbergensis*.

The effect of different concentrations of sodium hydroxide on the extraction of vitamin B_6 was examined by autoclaving dried bakers' yeast at 15 lb. pressure for 1 hour with 0·1 *N*, *N*, and 2 *N* solutions. A further series of samples was treated as before and hydrochloric acid added after autoclaving to bring the final concentration of acid to 0·055 *N*. The solution was again autoclaved at 15 lb. pressure for 1 hour. In all cases 5 g. of yeast and 40 ml. of the sodium hydroxide solution were used. Concentrated hydrochloric acid was used to bring the final acid concentration to 0·055 *N* in a total volume of 50 ml.

The results, given in Table VII, show that the maximal amount of vitamin B_6 was extracted by sodium hydroxide treatment alone. Further treatment of the sample with 0·055 *N* hydrochloric acid appears to be unnecessary when *N. sitophila* is used as the assay organism and the alkali at least 1·0 *N* in concentration.

TABLE VII

EFFECT OF DIFFERENT CONCENTRATIONS OF NaOH AND OF NaOH FOLLOWED BY 0·055 *N* HCl ON THE EXTRACTION OF VITAMIN B_6 FROM DRIED BAKERS' YEAST

µg. Vitamin B_6 per g. of dried yeast					
0·1 *N* NaOH		*N* NaOH		2 *N* NaOH	
Alone	Followed by 0·055 *N* HCl	Alone	Followed by 0·055 *N* HCl	Alone	Followed by 0·055 *N* HCl
13	11	10	11·8	10	10
16·3	11	11·3	10·8	10	12

The greater variation shown with 0·1 *N* sodium hydroxide treatment seems to be due to incomplete destruction of the aneurine present. Treatment with 0·055 *N* hydrochloric acid appears to destroy either any residual aneurine or some other factor affecting the assay.

A further series of tests with yeast was carried out to test the efficiency of the treatment and to determine whether concordant duplicate results could be obtained. Table VIII gives the results obtained.

TABLE VIII

ASSAY OF VITAMIN B_6 CONTENT OF BREWERS' YEAST, USING *N* NaOH TO INACTIVATE THE ANEURINE

Amount of final diluted sample added, ml.	µg. of Vitamin B_6 per g.		
	Yeast 2	Yeast 3	Yeast 4
1	17	19	23
2	16	—	—
3	17	—	—
4	20	21	26

The maximum variation is very much lower with sodium hydroxide than by the use of sodium sulphite. The sodium hydroxide treatment for the inactivation of aneurine has, therefore, been adopted for the assay of vitamin B_6.

8. EFFECT OF LIGHT ON THE ACTIVITY OF PYRIDOXINE—Daylight and artificial light are known to affect and inactivate pyridoxine, but only recently have results been published showing the exact effect. Cunningham and Snell (1945) have shown that a major inactivation takes place when a pyridoxine solution is exposed to direct sunlight for 1 hour, whereas diffuse daylight or artificial light give minimal losses for the same time of exposure. The present experiments deal with the effect of artificial light and ultra-violet light on pyridoxine activity.

In the first series a solution of pyridoxine, containing 0·2 μg. per ml., was exposed to ultra-violet light (1849–4500 A.) for 30 to 120 minutes. The source of light was placed 30 cm. from the solution, which was exposed directly in a crystallising dish to the ultra-violet light. The solution was 0·5 cm. in depth. After treatment the solution was assayed and in all cases the pyridoxine was completely inactivated within the sensitivity of the test. Using a solution containing 20 μg. per ml. exposed for 1 hour to ultra-violet light, a loss of activity of only 35 per cent. was recorded. The rate of inactivation would appear to depend, in part at least, on the pyridoxine concentration of the solution irradiated, other factors being kept constant.

The effect of artificial light was studied by exposing a solution of pyridoxine, containing 20 μg. per ml., to the rays from a 100-watt lamp for 4 hours under exactly the same conditions as for the ultra-violet light exposure. Only 6 to 10 per cent. of the pyridoxine appeared to be inactivated.

From the results it may be concluded that artificial light has little or no effect on the assay of pyridoxine, whereas direct sunlight or ultra-violet light must be avoided.

9. RECOMMENDED METHOD OF TREATING SAMPLES—Weigh 5 g. of the sample to be assayed into a flask and add 40 ml. of *N* sodium hydroxide. Autoclave the whole for 1 hour at 15 lb. pressure, cool, dilute with water to 100 ml. Pipette 10 ml. of the solution into a 50-ml. conical flask and neutralise to *p*H 7 with concentrated hydrochloric acid. Adjust the *p*H to 4·6 with *N* hydrochloric acid, using external indicators, and finally dilute the solution to give a concentration of approximately 0·3 μg. of vitamin B_6 per ml., filtering if necessary.

The method of assay is, otherwise, similar to that published by Stokes.

10. ASSAY OF VARIOUS FOODSTUFFS—The results of assays on different samples of brewers' yeast, milk, and various foods are given in Table IX.

TABLE IX

VITAMIN B_6 ASSAY OF BREWERS' YEAST, MILK, AND VARIOUS CEREALS AND CEREAL MIXTURES

Material	Number of samples assayed	Pyridoxine per g. of material
Dried yeast A	3	135
Pressed yeast A	1	20
” ” B	1	25
” ” C	1	14
” ” D	1	7
” ” E	1	6
” ” F	1	5·4
Dried whole milk	3	7
Fresh milk	2	6·5
Wheat	2	13
Maize meal	2	5·6
Oats	2	8·9
Dairy nuts	2	8·2
Poultry mash	2	9·6

The results obtained are considerably higher than the majority of previously published results by Stokes's original method, and this, it is felt, is due, in part at least, to the replacement of sodium sulphite and hydrogen peroxide by sodium hydroxide for the inactivation of the aneurine present in the samples. The considerable variation in the vitamin B_6 content of pressed brewers' yeast (all samples of which contained approximately 25 per cent. of total solids) may be due to the rather abnormal conditions at present existing in the brewing industry. Samples tested from weekly batches of yeast were found to vary considerably in vitamin B_6 content, as can be seen in Table IX.

SUMMARY

1. A study has been made of several factors that might possibly interfere with the assay of vitamin B_6 by means of the mould *N. sitophila*. Of these factors, the age of the culture has no effect on the response found.

2. In the assay of vitamin B_6 it has been found that aneurine must be inactivated, but this cannot be carried out with sodium sulphite and hydrogen peroxide, or pyridoxine will also be affected.

3. A method has been evolved in which the aneurine is inactivated by heat treatment in presence of *N* sodium hydroxide, and several assays of brewers' yeast, dried milk, and various foods by this method are recorded.

4. The effect of ordinary artificial light on pyridoxine is slight but ultra-violet light may cause total inactivation.

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Phosphate Removal in Qualitative Analysis

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OF the several methods proposed for the removal of the phosphate ion in qualitative analysis, most require a modification of, if not a complete departure from, the usual systematic scheme for cation separation effected in absence of the phosphate; moreover, they frequently demand not a little manipulative skill on the part of the operator for their successful execution, skill that can only be acquired by continual use. For those procedures that do not require subsequent rearrangement of the group scheme, the remark about manipulation applies to an even greater degree. However, of the two types, the latter are the more attractive, and, inasmuch as their disadvantages are confined to the technique of phosphate removal their improvement would appear to be possible by an improvement of that technique.

Accordingly, two of these methods have been examined—one, where the phosphate is removed by stannic chloride, and the other where it is precipitated by means of soluble zirconium salts.

STANNIC CHLORIDE METHOD

The procedure originally devised by Gatterman and Schindhelm[1] entails the precipitation of the phosphate by addition of stannic chloride to a slightly acid solution and removal of the excess of tin by sulphide precipitation. The main objection lies in the bulk and nature of the precipitate formed, which is colloidal and very difficult to filter.

Experimental—The use of tannin as a flocculating agent is well known, and the tin–tannin complex (recently described by Holness and Schoeller[2] for the quantitative separation of tin from aluminium, iron, manganese, etc. in hydrochloric acid solutions) is precipitated under the conditions of the removal; hence it was to be expected that tannin might prove to be of value in eliminating this filtration difficulty. But these expectations were only realised on addition of a considerable quantity of tannin, excess of which it is necessary to remove. Also, although complete removal of phosphate is not claimed by the authors of the method, it was observed that tannin actually increased the amount of residual phosphate, as detected by the molybdate test.

The use of solid stannic chloride pentahydrate, instead of the "solution" containing 5 g. of solid in 5 ml. of water,[3] also increased the amount of residual phosphate. Ammonium stannichloride, 5 g. of which were dissolved in the minimum of water (20 ml.), proved to be as efficacious as the stannic chloride, and it has the advantage of being non-hygroscopic. As with solid stannic chloride, the solid stannichloride failed to remove all of the phosphate in one operation. Addition of tannin had the same effect with it as with stannic chloride, *i.e.*, it caused even more incomplete removal than occurred without it.

The slow addition of the stannic chloride was tried, as recommended for zirconium phosphate (*infra*). Although this facilitated filtration to a certain extent, some phosphate remained in solution even after the operation had been repeated three times.

Discussion—The intractability of the precipitate and the variable extent of phosphate removal are serious factors operating against the applicability of this method. Reference to these drawbacks brings up the question of the constitution of the precipitate. It has been stated to be an adsorption complex of PO_4''' on stannic acid, and this is borne out by the fact, mentioned above, that tannin is able to break up this complex to a certain extent to form the tin-tannin complex. This is not to be expected of a definite compound. Considerable experimental evidence has been adduced in support of the general theory that tannin acts by flocculation of a disperse hydroxide,[4] and the observation that the phosphate is precipitated by the stannic chloride solution over the same acidity range as that at which tannin precipitates tin would suggest that the action of stannic chloride on the phosphate is the result of its hydrolysis. This further points to the formation of a hydroxide-phosphate complex.

ZIRCONIUM PHOSPHATE METHOD

Of all the metallic phosphates, those formed by zirconium and hafnium are the least soluble in strong acids. Use of this property in phosphate separation was first made by Curtman and his co-workers,[5,6] who added a zirconyl chloride solution to an acid solution of the interfering phosphate. More recently, the use of zirconyl nitrate has been proposed.[7]

The chief disadvantages of the original method are:

(*a*) the precipitate formed is colloidal,
(*b*) there is a large loss of cations from solution,
(*c*) phosphate removal is often incomplete,
(*d*) excess of zirconium interferes with the subsequent tests for aluminium, and
(*e*) an extra separation is entailed—that of zirconium in Group IIIa.

One modification[8] involves the use of more dilute zirconium solutions but does not overcome (*a*), (*c*), (*d*), and (*e*). Reilly and O'Brien[9] have recognised that (*a*) is due to the presence of a large excess of zirconium, and suggest the slow, drop by drop, addition of the zirconyl solution as being effective in producing a filterable precipitate. Their method, although an improvement, still does not obviate (*d*) and (*e*).

EXPERIMENTAL—

Tannin was again employed in the investigation of these objections, using the zirconium-tannin complex (described by Schoeller[10] for the quantitative separation of zirconium from aluminium, iron, manganese, etc.). The reagent was very effective in producing a filterable precipitate; removal of excess of tannin was effected by addition of a few drops of zirconium solution to the filtrate, but the resulting solution contained some zirconium. Attempts to precipitate this with saturated potassium sulphate and with potassium ferrocyanide were made, but they were unsuccessful for the small quantities of zirconium present, since they merely formed a cloudiness in the solution on standing for some time and this could not be removed by filtration. Rendering the zirconium non-interfering as fluozirconate was also tried, equally without success. The method was no improvement on that of Reilly and O'Brien.

In order to remove the excess of tannin, resort was made to the tin-tannin complex again, by addition of ammonium stannichloride, with subsequent removal of residual tin with hydrogen sulphide. This was found to be very satisfactory: the successive steps prevent any interference that may result from the presence of zirconium, tannin, or tin.

PROCEDURE—

The following details apply to solutions containing up to 0·7 g. of phosphate ion. Transfer the filtrate from Group II to a 100-ml. beaker, boil to remove hydrogen sulphide, and test a small portion for the presence of phosphate with ammonium molybdate. If positive, add 2 g. of ammonium chloride, boil, and add slowly 10 ml. of *either* an 8 per cent. aqueous solution of zirconyl chloride *or* a 7 per cent. aqueous solution of zirconyl nitrate, and boil again. Add diluted ammonia solution (1 + 1) until the solution is just alkaline to litmus, followed by *N* hydrochloric acid until it is just acid and boil. Add 10 ml. of 1 per cent. tannin solution, boil and add 10 ml. of a 1 per cent. solution of ammonium stannichloride in 0·5 *N* hydrochloric acid. Boil, filter, and discard the precipitate. Test a portion of the filtrate for the presence of phosphate ions.

If the test is negative, pass H_2S into the remainder of the filtrate, filter (rejecting the precipitate), boil off hydrogen sulphide from the filtrate, oxidise any ferrous iron with concentrated nitric acid and precipitate the iron group by addition of ammonia in the usual way.

In order to obtain some estimate of the loss of ions occasioned by the separation, figures were obtained for the recovery of a fixed amount of cation from solutions containing varying amounts of phosphate. It was thought that iron, aluminium, zinc, and barium would be representative of the metals of Groups IIIa, IIIb, and IV, and they were investigated as follows:

Iron—A soln. of ferric alum was made up, and standardised by precipitation as hydroxide and ignition to Fe_2O_3. Seventy-five-ml. portions were withdrawn, A.R. diammonium hydrogen phosphate was added, the separation as described above effected, and the iron determined as Fe_2O_3.

Aluminium—Three 50-ml. portions of a stock soln. of ammonium alum were treated with differing amounts of A.R. diammonium hydrogen phosphate, the phosphate was removed as described and the aluminium recovered as Al_2O_3.

Zinc—Separations were made on three solns. of zinc sulphate, each containing a known amount of zinc and A.R. diammonium hydrogen phosphate, after which the zinc was determined by titration against a standard potassium ferrocyanide solution.

Barium—The barium content of 50 ml. of a solution of barium chloride was determined as $BaSO_4$. To three other equal volumes of the same solution were added different weights of A.R. diammonium hydrogen phosphate; removal of the PO_4''' ion was carried out as above and the barium again determined as $BaSO_4$.

No.	Phosphate added g. $(NH_4)_2HPO_4$	Cation taken g.	Cation found g.	Recovery %
1	0·05	0·284 Fe_2O_3	0·277 Fe_2O_3	97·5
2	0·20	0·284 ,,	0·268 ,,	94·0
3	0·60	0·284 ,,	0·255 ,,	92·0
4	1·00	0·284 ,,	0·236 ,,	83·0
5	0·05	0·193 Al_2O_3	0·182 Al_2O_3	94·5
6	0·50	0·193 ,,	0·169 ,,	87·5
7	1·00	0·193 ,,	0·154 ,,	80·0
8	0·05	0·072 Zn	0·071 Zn	98·0
9	0·50	0·072 ,,	0·068 ,,	93·5
10	1·00	0·072 ,,	0·060 ,,	83·0
11	0·05	0·252 $BaSO_4$	0·246 $BaSO_4$	98·0
12	0·50	0·252 ,,	0·244 ,,	97·0
13	1·00	0·252 ,,	0·241 ,,	96·0

DISCUSSION—

The original procedure as first proposed was undoubtedly unsatisfactory, but it has been substantially improved in various ways by later workers. The most significant advance has been that of Reilly and O'Brien,[9] but their method of removing the surplus zirconium by boiling in alkaline solution leaves much to be desired. In the revised procedure given above, the system is such that each successive reagent removes excess of the former, and interference is prevented. Thus the phosphate is removed by a zirconium solution, excess zirconium is removed by tannin (which also serves as a flocculating agent), and excess of tannin is removed by tin. Excess tin is removed by H_2S. The average time for the complete sequence of operations is 15 minutes.

A further advantage of this scheme is that different amounts of phosphate are removed with equal ease, since the method is a "sliding scale" one. A small amount of phosphate leaves a large excess of zirconium, which is removed as described, leaving a comparatively large excess of tin, which is conveniently precipitated as sulphide. Similarly, a large amount of phosphate leaves a small quantity of tin, which is also dealt with easily by the H_2S. The recovery figures quoted show that for qualitative purposes, the loss in cations is not great.

One apparent objection would seem to be the expense of the zirconium salts. Actually, however, the quantities used are such as to make the method cheaper than that using stannic chloride: 5 g. of $SnCl_4.5H_2O$ are used for every operation by the stannic chloride method, whereas only 0·8 g. of $ZrOCl_2.8H_2O$ and 0·1 g. of $(NH_4)_2SnCl_6$ are used in the revised method described.

The modified procedure has become standard practice in these laboratories, and has been in satisfactory use for many months.

SUMMARY

Two methods for the removal of the phosphate ion in qualitative analysis have been examined and discussed, *viz.*, the stannic chloride and the zirconium phosphate methods. It is seen that, of the two, the latter has the wider application. A modified procedure has been outlined whereby the rapid and complete removal of quantities up to 0·7 g. of phosphate may be effected whether it be present as the major or the minor constituent of the sample

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The Quantitative Analysis of Crude and Refined Carbazole

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THE growing demand for carbazole as an intermediate for the preparation of a number of technically useful derivatives has made a re-examination of the various extant or proposed methods for estimating the carbazole content of materials of coal tar origin desirable. Coal tar contains a relatively high proportion (about 2 per cent.) of carbazole and is not likely to be displaced as its source. The closest congeners of carbazole in tar are anthracene and phenanthrene and they are therefore the chief contaminants of crude and refined specimens of carbazole submitted to analysis. Besides these, a number of other substances, chiefly aromatic hydrocarbons but not excluding heterocyclic nitrogen compounds, may be present to a lesser extent.

Of the older analytical methods[1] only one based on an estimation of the nitrogen content (which is assumed to be wholly due to carbazole) by Kjeldahl's process has been used extensively. This has been said[2] to yield results unduly high by 2 to 4 per cent., but our experience with material known to be high in carbazole was quite the reverse. If the method is employed on very crude materials, containing nitrogenous compounds other than carbazole, errors of unknown magnitude and direction must arise. However, the main drawback to the method is that carbazole is extremely resistant to decomposition by the Kjeldahl process and specimens require 20 to 30 hours digestion before clear and colourless solutions suitable for ammonia estimation are secured. This prolonged heating is not a direct cause of loss of ammonium salts and the consequent low analytical results.

Two other methods[1] depended upon (*a*) the quantitative separation of carbazole as the N-potassium derivative, which was decomposed and the recovered carbazole weighed, and (*b*) a quantitative bromination. The first has no feature to recommend it, and although Ardashev[2] preferred the bromination method to the Kjeldahl nitrogen estimation, it does require a supplementary independent estimation of the anthracene content (anthracene, if present, is also brominated), and a very close control of experimental conditions is needed, as he showed, to define exactly the number of molecules of bromine (2 or 3) that react with the carbazole. Neither method was thought sufficiently attractive to warrant any further examination.

Cohn[3] suggested that a method of analysis could be based on the acetylation of carbazole, excess of acetic acid being titrated. Some preliminary experiments on these lines were not promising. The presence of N-acetyl carbazole in the mixture to be titrated is very inconvenient and the method was not persevered with.

Recently, Chmelevsky and Postovsky[4] have proposed a method based on the quantitative nitrosation of the carbazole in specimens derived from coal tar. This has the merit of being rapid and is not interfered with by any anthracene or phenanthrene present. The specimen is treated with nitrous acid, generated from sodium nitrite and acetic acid, and the excess of nitrous acid is decomposed to produce nitric oxide (NO), which is swept out in a stream of carbon dioxide into strong caustic potash solution and its volume measured. The technique of this method has now been closely studied and it has been found that when adequate precautions are taken, as detailed below, the results with purified and crude carbazole specimens and with mixtures of purified carbazole, anthracene and phenanthrene are reproducible.

1. The carbon dioxide used for sweeping out air initially, and nitric oxide finally, from the reaction flask, was generated from marble chips, broken small and kept beneath boiling water for 2 or 3 hours before use, and recently boiled aqueous hydrochloric acid (equal volumes of concentrated acid and distilled water). It was dried by bubbling through concentrated sulphuric acid before passage into the reaction flask.
2. The specimen for analysis was placed in the reaction flask and dissolved in a fixed amount of chlorobenzene and acetic acid. The acetic acid, previously distilled from chromic acid, was freed from peroxides before use by boiling for 30 minutes under reflux with cupric acetate and then distilling. The chlorobenzene - acetic acid solution was heated just to boiling to expel dissolved air.
3. The purity of the sodium nitrite was checked by titration with standard potassium permanganate solution and a correction was determined, from blank experiments, for the residual amount of nitric oxide dissolved in the reaction mixture.
4. The reaction mixture was warmed to 40° C. towards the end of the evolution of nitric oxide.
5. The nitric oxide collected was not transferred to a graduated tube and measured over water but measured in the nitrometer after allowing time for it to reach room temperature. When nitric oxide is transferred over water an appreciable but indeterminate amount is lost by solution. For example, in an experiment in which 22·2 ml. at N.T.P. were measured over 50 per cent. caustic potash solution the amount after transference diminished to 21·3 ml. at N.T.P. Nitric oxide is about three times as soluble in water at room temperatures as nitrogen.

If the precautions detailed above are neglected considerable errors arise in both directions and the reproducibility of the analytical results is much reduced. Thus if air is not eliminated from the system and if the acetic acid contains peroxide some of the nitric oxide is oxidised to nitrogen peroxide (NO_2) and lost, but the volume of gas collected will be contaminated with nitrogen. This generally leads to low results for the carbazole content of a specimen. On the other hand, if the nitric oxide is not fully swept out, and the residual amount dissolved is not allowed for, results will be high. A further source of variability is incomplete nitrosation. Our results with highly purified specimens of carbazole showed that under the conditions laid down about 4 per cent. escaped nitrosation. This was allowed for in computing the carbazole content of specimens submitted to analysis.

The technique recommended reduces the uncertainty in a single estimation to ± 0·6 per cent. with a carbazole specimen upwards of 90 per cent. purity, and to ± 0·2 per cent. if the carbazole content is about 30 per cent.

EXPERIMENTAL

CRUDE AND PURIFIED CARBAZOLE SPECIMENS—

Samples of carbazole from three sources were used in this work.

Specimen A—A specimen of "pure carbazole" separated from coal tar and of German origin had m.p. 243° to 245° C.

Specimen B—Specimen A was recrystallised three times from chlorobenzene to constant m.p. 244·5° to 245·5° C. (corrected) and taken as being then sufficiently pure to use as a standard, *i.e.*, its purity was taken as nominally 100 per cent.

Specimen C—A sample of synthetic carbazole was prepared from the phenylhydrazone of *cyclo*hexanone through tetrahydrocarbazole,[5] the latter being dehydrogenated by boiling a solution in xylene with chloranil.[6] The crude synthetic carbazole was recrystallised from chlorobenzene to m.p. 244° to 245° C. (corrected).

Specimen D—A crude crystalline fraction containing about 30 per cent. of carbazole was obtained from a firm of tar distillers. This was fully representative of material derived from coal tar at an early stage in the separation of carbazole. It was somewhat oily and the analytical results reported herein seem to show that it contained nitrogenous substances additional to carbazole. Specimens taken for analysis were from a portion that had been thoroughly ground and mixed to render it homogeneous.

ESTIMATION OF NITROGEN CONTENT IN CARBAZOLE-CONTAINING MATERIALS BY KJELDAHL'S METHOD—

The method adopted followed orthodox lines.[7] The specimen, containing about 0·8 g. of carbazole, was digested with 23·5 ml. of concentrated sulphuric acid containing 0·5 g. of copper sulphate crystals, 0·1 g. of selenium and 16 g. of potassium hydrogen sulphate. A further 10 ml. of concentrated sulphuric acid were added during the digestion. Generally from 15 to 30 hours were allowed, this being 2 or 3 hours additional to the time required for the disappearance of organic coloration.

Duplicate results on Specimen A were 7·63 and 7·70 per cent. of nitrogen, from which the carbazole content, assuming all nitrogen found arises from carbazole, is 91·0, 91·9 per cent. In a mixture of this specimen with equal weights of pure phenanthrene and anthracene, the nitrogen content was found to be equivalent to a weight of carbazole equal to 91·7 per cent. of the weight of Specimen A taken.

Similar duplicate results on Specimen B (the standard specimen) corresponded to 91·9 and 92·6 per cent. for the carbazole content.

Specimen D gave the following figures for carbazole content:—31·6, 31·1, and 31·2 per cent.

Additionally, three further experiments were carried out. The first, a blank estimation (*i.e.*, without introduction of carbazole but in which the digestion was continued for 4 hours) showed nitrogen, 0·0 per cent. The other two were designed to estimate any loss of ammonium salts due to the lengthy digestion period. First, a weighed amount of ammonium sulphate was added to the Kjeldahl reagents and immediately estimated in the usual way: 99·3 per cent. of the weight taken was found, which may be taken as an estimation of the purity of the ammonium salt. Then the experiment was repeated, but 16 hours digestion was allowed before estimation of the ammonium salt. The amount found was now 98·9 per cent. of that taken. The difference in the two results (less than 0·5 per cent.) is small enough to show that the larger loss of nitrogen occurring in actual carbazole estimations does not take place after conversion to ammonium sulphate.

QUANTITATIVE NITROSATION OF CARBAZOLE-CONTAINING MATERIALS

OUTLINE OF RECOMMENDED METHOD—

The apparatus employed is shown diagrammatically in Fig. 1. A weighed amount of the specimen for analysis (containing about 0·5 g. of carbazole) is placed in the reaction flask (F) and dissolved in a mixture of 20 ml. of chlorobenzene and 20 ml. of acetic acid by heating to boiling. After cooling, the flask is attached to the rest of the apparatus: the sodium nitrite (about 0·33 g.), weighed accurately in its container (D), is previously introduced into the inlet tube. A vigorous stream of carbon dioxide is passed for about 20 minutes and the rate then reduced to 2 to 3 bubbles per second, when it will be found, by the usual test of filling the nitrometer with potash, that the volume of undissolved gas collected is negligible (*i.e.*, less than 0·1 ml. in 10 mins.). The sodium nitrite container is allowed to fall and its contents are mixed, with occasional shaking, into the contents of the flask. After about 30 minutes, when evolution of nitric oxide has almost ceased, the reaction flask is immersed in a water bath maintained at 40° C. for about 15 minutes and shaken from time to time, after which no appreciable further increase in the volume of nitric oxide in the nitrometer (G) will occur. The potash reservoir is raised so that the liquid levels are equal, and between 30 minutes and 1 hour allowed for the gas to assume room temperature before final measurement of its volume and temperature and the barometric pressure.

Calculation of carbazole content—The calculation is based upon the following equations:

$$C_{12}H_9N + NaNO_2 + CH_3COOH = C_{12}H_8N_2O + CH_3COONa + H_2O$$

$$3NaNO_2 + 2CH_3COOH = 2NO + NaNO_3 + 2CH_3COONa + H_2O.$$

In one estimation, 0·5042 g. of pure carbazole reacted with 0·3290 g. of sodium nitrite (purity estimated by titration 99 per cent.) and the volume of nitric oxide collected was 27·8 ml. at 20·5° C. and 766·8 mm., corresponding to 26·09 ml. at N.T.P. A standard correction of 1·0 ml. to allow for dissolved nitric oxide (see below) is added, making 27·09 ml. Hence, weight of carbazole nitrosated =

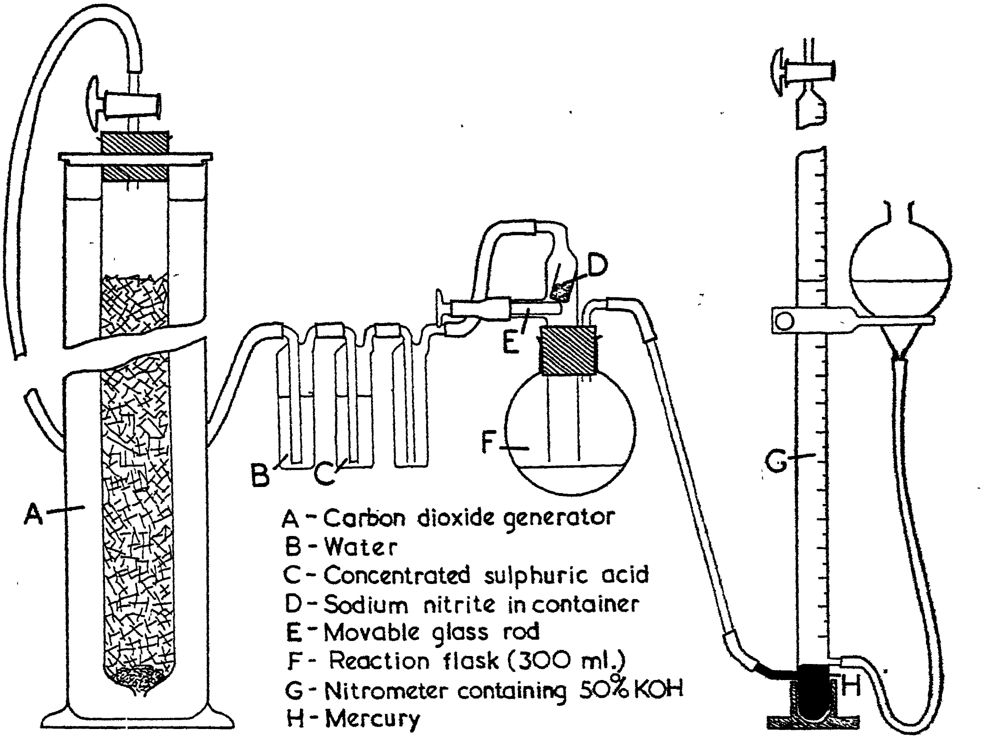
A
B
C
D
E
F
G
H
A - Carbon dioxide generator
B - Water
C - Concentrated sulphuric acid
D - Sodium nitrite in container
E - Movable glass rod
F - Reaction flask (300 ml.)
G - Nitrometer containing 50% KOH
H - Mercury

Fig. 1

$$\frac{167}{69}\left(0{\cdot}3290 \times \frac{99}{100} - 27{\cdot}09 \times \frac{3}{2} \times \frac{69}{22400}\right) = \frac{167}{69} \times 0{\cdot}2005,$$

which is increased by a factor 1·04 to allow for incomplete nitrosation (see later, p. 50), so that the carbazole content is in

$$\frac{167}{69} \times \frac{0{\cdot}2005}{0{\cdot}5042} \times 1{\cdot}04 \times 100 = 100{\cdot}1 \text{ per cent.}$$

ESTABLISHMENT OF THE RECOMMENDED METHOD

Correction for solubility of nitric oxide—

Six identical experiments were made in which sodium nitrite in quantity sufficient to furnish about 26 ml. of nitric oxide was decomposed in the apparatus in absence of carbazole. The sodium nitrite was 99 per cent. pure by titration: from the amounts of nitric oxide collected, the purity was calculated to be 95·0, 94·5, 94·8, 94·6, 95·6 and 95·5 per cent.; mean 95·0 per cent., standard deviation for a single estimation 0·46 per cent. When the standard correction 1·0 ml. was added to the gas volumes after reduction to N.T.P., the mean of the calculated values for the purity of the sodium nitrite became 99·1 per cent. These experiments indicate the preferability of estimating the nitrite by titration rather than by a single blank experiment, and they justify the correction of the volume collected by the standard addition of 1·0 ml. for the solubility of nitric oxide in the reaction medium.

Estimations on carbazole-containing material—Correction for incomplete nitrosation—

Six identical experiments on carbazole Specimen B, using sodium nitrite in excess sufficient to provide the same volume of nitric oxide as in the blank experiments, gave the following results after applying the standard correction to the volume of gas collected: 96·7, 95·6, 96·8, 95·5, 96·2 and 96·2 per cent. (mean 96·2 per cent. standard deviation 0·54 per cent.).

If the carbazole percentages as found are multiplied by 1·04, the mean is now 100·0 per cent. and standard deviation 0·6 per cent., and it therefore appears legitimate to allow for incompleteness in nitrosation in this way.

The carbazole Specimen C was found in three estimations to have the purity 99·8, 99·8 and 99·6 per cent. (mean 99·7 per cent.) in terms of Specimen B = 100 per cent.

Two estimations on the cruder carbazole (Specimen D) gave carbazole content 28·4, 28·0 per cent., mean 28·2 per cent. Compared with the results of the Kjeldahl estimation (mean of three, 31·3 per cent.), they appear low, and as the Kjeldahl method applied to purer carbazole specimens (A and B) gave markedly lower results than the nitrosation method, it must be supposed that the cruder material contains nitrogen compounds other than carbazole that are not nitrosated.

Experiments were next made with mixtures containing only carbazole, anthracene, and phenanthrene. In a mixture of commercial "pure" anthracene and phenanthrene, containing roughly equal amounts by weight, the carbazole content was found to be 0·24 per cent. When to this mixture approximately half its weight of carbazole (Specimen B) was added, the carbazole found was 32·7 per cent., against 32·5 per cent. calculated.

In order to make more certain that the figures obtained for the carbazole content of crude material (Specimen D) by the nitrosation method are more reliable than those obtained through the Kjeldahl nitrogen estimation, duplicate experiments were made in which a mixture of Specimens D and B was analysed. The results were—Found in the mixture 0·5141 and 0·5063 g. of carbazole; Calculated from prior analyses 0·5135 and 0·5058 g., *i.e.*, weight found = 100·1 per cent. of weight calculated in each instance. These experiments show clearly that in the nitrosation method it is the actual carbazole content that is determined and impurities in crude specimens, whether nitrogen-containing or not, do not interfere with the method.

Additional experiments—

The use of a mixture of chlorobenzene and acetic acid as the reaction medium has some advantage. If acetic acid is used alone, the carbazole is largely undissolved at the beginning of the reaction with nitrous acid, and nitrosation tends to be less complete, giving lower results. When a mixture of acetic acid with a small proportion of acetic anhydride was used in an effort to make the nitrosation more complete, by removal of water produced in the reaction itself, the opposite result was found and was evidently due to preferential acetylation of the carbazole. An increase in the proportion of sodium nitrite with a corresponding diminution in the weight of specimen analysed effected no improvement.

In two blank experiments sodium nitrite was decomposed first in acetic acid and then in the same volume of a mixture of chlorobenzene and acetic acid (in equal volumes—the proportions finally adopted). It was found that the nitric oxide collected in the latter case corresponded stoichiometrically more closely to the sodium nitrite taken (97·0 and 98·2 per cent). and it is supposed that this is due to a lessened solubility of nitric oxide in the mixture. In these estimations the reaction mixture was heated finally to 50° C. Choice of 40° C. as the temperature to be adopted was made because in an actual carbazole estimation on Specimen B in which the temperature was raised to 85° C., the nitrosate visibly decomposed with evolution of gas. The correction for dissolved and unswept nitric oxide is not large if the mixture is heated to 40° C., which is a convenient temperature to standardise.

When the amount of acetic acid was reduced to 3 ml. in 70 ml. of chlorobenzene (an amount of acid which is still 10 mols. per mol. of sodium nitrite), reaction between it and the sodium nitrite was impracticably slow.

SUMMARY

It has been shown that crude and refined carbazole specimens can be analysed by quantitative nitrosation with convenience and accuracy. The uncertainty can be reduced to ± 0·6 per cent. on an individual estimation with a purified carbazole specimen, and less, ± 0·2 per cent. (calculated on the sample), with a crude specimen containing about 30 per cent., if a number of detailed precautions are taken.

Thanks are due to Mr. D. W. Milner of Yorkshire Tar Distillers Ltd. for the sample of crude carbazole.

The work described above has been carried out as part of the research programme of the Chemical Research Laboratory, and this paper is published by permission of the Director of the Laboratory.

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June, 1948

Notes

CALCIUM TARTRATE CRYSTALS IN PROCESSED CHEESE

To investigate a complaint that a certain type of cheese was being sold containing "bits of glass," a specimen was obtained from a "5-lb." block of cellophane-wrapped imported processed cheese; and on making a cross-cut with a knife, at least one of the particles in question was usually visible on the newly-exposed surface. The particles were immediately recognisable as crystals and were remarkably perfect as to the facets; but all had milky opaque spots at or near the centre due to inclusions. The crystals were fairly uniform in size, and their over-all dimensions were about 0·9 mm. × 0·9 mm. × (1·5 to 2 mm.). Fig. 1 gives the side and end view of a well developed crystal. The almost square end-view was typical of every crystal seen, but the development of the pyramidal facets varied, so that the edge corresponding to *ab* could be long or short. The small triangular facets *w, x, y, z*, placed alternately at the two ends of the crystal, were always seen and were relatively larger in the smaller crystals. The inclusions, Fig. 2, were well shown by immersing a small crystal in clove oil in a hollow-ground slide. The crystals were found to contain tartrate and calcium.

The 11th World's Dairy Congress, 1937, recommended that no more than 3 per cent. of emulsifying salts should be permitted in processed cheese.[1] A standard of "not more than 3 per cent. of harmless emulsifying agents" is in force in New South Wales.[2] For the purposes of the Processed Cheese-Order, 1942, processed cheese was defined as a cheese which after manufacture has been heated and to which an emulsifying agent has been added. Under the Labelling of Food Orders 1944 and 1946, processed cheese is exempted from the requirements as regards declaration of ingredients. J. R. Nicholls[3] and H. E. Cox[4] mention disodium phosphate, sodium citrate and sodium potassium tartrate as being the commonest emulsifiers.

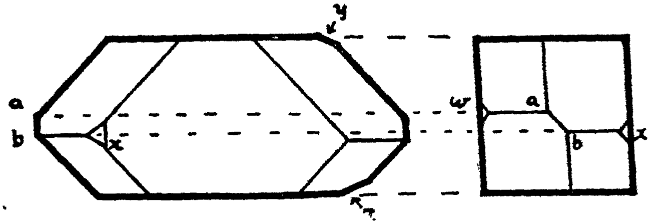

Fig. 1

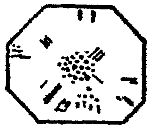

Fig. 2

In the present instance it appears likely that a tartrate has been added as an emulsifier during manufacture and that it has reacted with the calcium present in the residual whey incorporated in the cheese. Calcium tartrate crystallises well on the microscope slide in prismatic crystals, which have long been known as a micro-test for calcium or tartrates.

The inclusions lend some support to the view that the crystals may have grown *in situ* in the cheese, and the perfection of the facets would indicate slow growth. The presence of these crystals is to be regarded in my view as a blemish or defect in quality, but would not render the cheese injurious to health.

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January, 1947

A DIAPHRAGM PUMP FOR AIR AND OTHER GASES

In microchemical operations a stream of low-pressure air finds numerous applications, *e.g.*, to assist evaporation,[1] to free solutions from dissolved gases,[2] and as a stirring agent in micro-titrations.[3] For the small rates of air flow required for such purposes, the electrically-operated diaphragm pump is well suited. Such devices have long been used in the aeration of aquaria, and the like; the modification here described is inexpensive and of simple construction.

As shown in Fig. 1, the device is actuated by an electric bell movement. By unscrewing the pump mechanism retaining screw A and then replacing the gong, the bell may be restored to its original form. The pump assembly, B, is constructed mainly from a thistle funnel and is held by its stem in clamp C. The latter is of sheet metal and lined with sheet rubber or cork. Stretched over the mouth of B and secured by a few turns of thread is a diaphragm, D, of sheet rubber cut from an old cycle inner tube. The position of B in the clamp is adjusted so that the centre of the diaphragm is sharply depressed when the hammer of the bell moves inwards. Air is then forced out through upper valve E, and the elasticity of the diaphragm causes it to follow the hammer on the return stroke, thus drawing in air through lower valve F. The cycle of operations is repeated as long as the current flows, so that a virtually continuous stream of air passes through the apparatus.

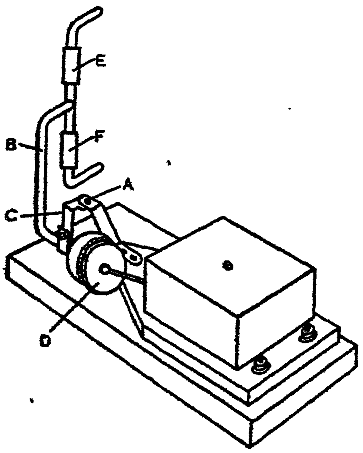

Fig. 1

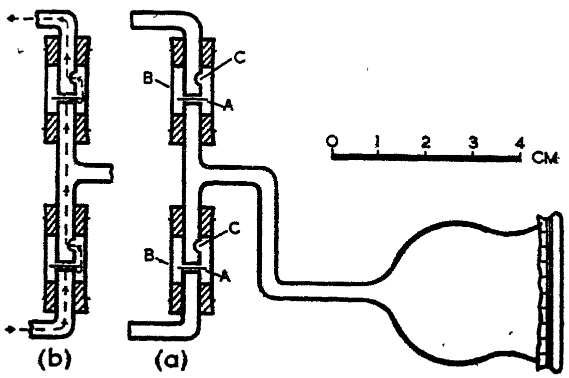

Fig. 2

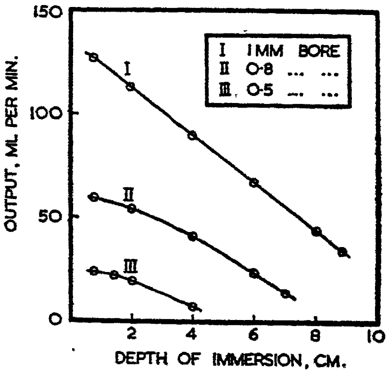

Fig. 3

Details of the pump mechanism are shown at (*a*) in Fig. 2. Valves A A are discs of thin sheet rubber cut by means of a wetted corkborer and are about 1 mm. smaller in diameter than the bore of valve chambers B B. Owing to the high impulse frequency, the valve-lift should be small; about 1 mm. is suitable. A manometer attached to the pump outlet is useful in determining the most suitable lift. Unidirectional flow of air, as shown at (*b*) by broken lines, is secured by blowing small holes C C in the valve-stops.

Performance naturally depends on the power of the bell mechanism and on various other factors. Results shown in Fig. 3 are merely typical and were obtained by measuring the rates of delivery of free air from various capillaries immersed in water. The delivery may be controlled by means of a screw clip or a fine-control stopcock.[4]

Another use for the pump is for the circulation of gases in a closed system; no metal comes into contact with the gases and leakage, other than by diffusion through the rubber, cannot occur. Provided the resistance is not too great, the device also functions effectively as an aspirator. We have used it in this way for the generation of hydrogen sulphide for micro-qualitative analysis.[5]

To produce a pressure higher than that obtainable with the diaphragm pump the complete valve assembly (*b*, Fig. 2) may be attached by its side tube to vent tube H of the micro-stirrer unit previously described.[6] In this manner pressures up to about 50 cm. of water are obtainable.

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THE COLORIMETRIC DETERMINATION OF TRACES OF BISMUTH IN LEAD

Colorimetric methods for the determination of traces of bismuth in lead have been described by Robinson[1] and by Zischkau.[2] Robinson showed that his method, which involves removal of most of the lead as chloride, separation of the bismuth by co-precipitation with ferric hydroxide and final colorimetric determination as iodide, gave satisfactory recovery of bismuth added to pure lead in amounts from 0·0005 per cent. to 0·002 per cent. Zischkau has described two methods: one, which is applicable to lead containing from about 0·003 per cent. to 0·01 per cent. of bismuth, is open to serious criticism because it involves separation of lead as sulphate, and was found by the present authors to be unsatisfactory; the other, for quantities of bismuth from about 0·005 to 0·1 per cent., uses thiourea for final colorimetric determination and is simple and direct, the lead not being removed.

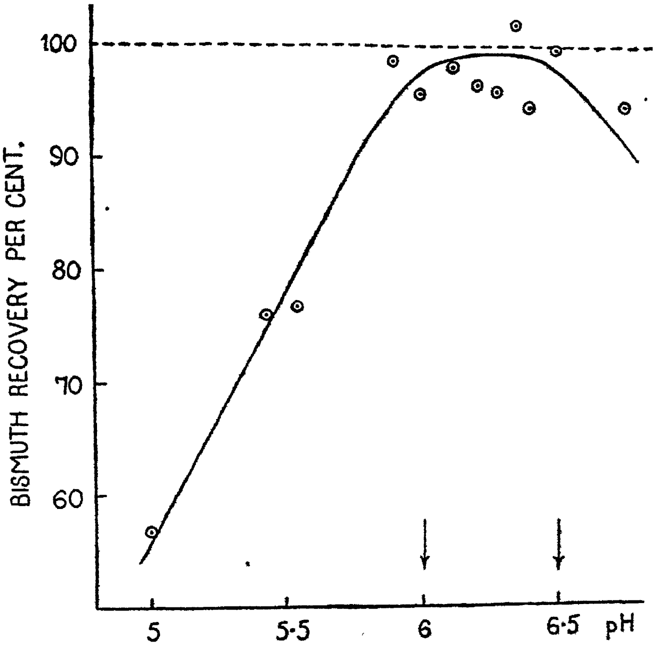

Fig. 1. Effect of *p*H of solution before filtering on the recovery of bismuth, showing optimum *p*H range.

When Robinson's method and Zischkau's second method were applied, using the Spekker photoelectric absorptiometer, to samples of lead used in chemical plant discordant results were obtained (Table I). In Robinson's method difficulty was experienced in adjusting the reaction of the solution, prior to filtering off the ferric hydroxide, owing to a blue colour obscuring the precipitated lead hydroxide acting as indicator. In establishing conditions for the separation of bismuth with ferric hydroxide, Robinson used the equivalent of only 0·0015 per cent. of bismuth but by determinations on lead with known amounts of bismuth added it was found by the present authors that Robinson's method is not entirely reliable for amounts of bismuth higher than about 0·002 per cent., low recoveries often being obtained.

The effect of *p*H at this stage was studied by making determinations on lead with added bismuth equivalent to 0·0045 per cent., the ferric hydroxide being filtered off from solutions of various *p*H values determined by glass electrode. Fig. 1 shows that the optimum *p*H range is 6 to 6·5. Following Robinson's original method it was found that the *p*H could be as low as 5 and in such cases the filtrate was brown and on standing several hours deposited a flocculent precipitate containing more bismuth. However, even at *p*H 5 satisfactory recovery of 0·0045 per cent. of bismuth was obtained if the precipitated ferric hydroxide was coagulated by boiling before filtration, the filtrate then being colourless and yielding no precipitate on standing.

TABLE I

Sample	Zischkau's method Bi %	Robinson's method Original Bi %	Robinson's method Modified as suggested Bi %
1	0·003	0·0025	0·0035
2	0·0033	0·0015	0·003
3	0·0025	0·002	0·0023

It appears therefore that for more than about 0·002 per cent. of bismuth the solution should be boiled before filtration or in cases where coloured solutions are encountered the *p*H should be previously adjusted to from 6 to 6·5, and that in any event by adjusting to this *p*H range correct results may be obtained on material containing up to 0·0045 per cent. of bismuth without repeating the determination on a smaller sample.

From Table I it is seen that results by the modified method agreed satisfactorily with those by Zischkau's method.

Thanks are due to the directors of May and Baker, Ltd., for permission to publish these results.

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July, 1948

TITRIMETRIC ESTIMATION OF FLUORINE

A NEW method for the titrimetric estimation of micro-quantities of fluorine was suggested by Milton, Liddell, and Chivers in 1942.[1] It involved the use of the sodium salt of sulpho-dichlorohydroxy dimethylfuchsin dicarboxylic acid (Solochrome Brilliant Blue B.S., Colour Index 723) in place of the alizarinsulphonate indicator normally used.

It was claimed that the method proposed allowed of a much sharper endpoint and gave a higher degree of precision than the alizarin technique.

Owing to the fact that the stocks of the new indicator soon became exhausted and it was withdrawn from the I.C.I. dyestuff catalogue, many workers anxious to utilise the method were unable to obtain supplies.

A dyestuff of similar composition is now made available by the Geigy Company under the name of Chrome Azurol S.[2] This indicator has been extensively tried out in the writer's laboratory for the past year and has been found to be quite as satisfactory as the original Solochrome Blue in micro-fluorine determinations. The method has been applied to the estimation of fluorine in atmospheres, soils, waters, bones and teeth, foodstuffs, vegetation including grass, blood, and urine, and it is confirmed that exceedingly precise results are obtainable.

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R. F. MILTON
October, 1948

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

South African Fish Products. Part XXVI. Application of the Fitelson Method of Squalene Determination to some Marine Oils. M. L. Karnovsky and W. S. Rapson (*J. Soc. Chem. Ind.*, 1947, **66**, 124–125)—The method described by Fitelson (*J. Assoc. Off. Agric. Chem.*, 1943, **26**, 499; 1945, **28**, 282: *cf.* Drummond and Thorbjarnarson, *Analyst*, 1935, **60**, 23) for determining unsaturated hydrocarbons in the unsaponifiable matter of fats has been applied to several marine oils.

Method—The unsaponifiable matter was separated by the S.P.A. method, four extractions being made. For the estimation of squalene, the directions of Fitelson were followed exactly, except that 2 g. of oil usually provided sufficient unsaponifiable matter for at least one determination. The following quantities gave satisfactory results in an alumina column 10 × 0·8 cm.:

Unsaponifiable fraction high in content of	Weight of material used per chromatogram	Volume of reagent used
Fatty alcohols	Not more than 120 mg.	10 ml. probably sufficient
α-Glyceryl ethers	„ „ „ 200 „	10 „ „ „
Sterols	„ „ „ 200 „	10 „ „ „
Vitamin-A	About 50 to 60 mg.	10 „ „ „
Unsaturated hydrocarbons	„ 25 to 35 „	15 to 20 ml.

With oils containing considerable amounts of vitamin A some adjustment of the amount of material used was necessary in order to retain a rapidly moving yellow band. The error involved in allowing the yellow band to pass into the filtrate is small where the method is used to estimate more than traces of unsaturated hydrocarbon.

A selection of the results obtained is given below.

Specific name	Common name	Oil from	Unsaponifiable matter, per cent.	Per cent. unsaturated hydrocarbon calculated as squalene in oil
Physeter macrocephalus	Sperm whale	head	47·28	0·12
Congermuraena australis	Cape eel	liver	11·80	0·18
Lophius piscatorius	Angler fish	„	2·62	0·12
Galeorhinus canis	Soup-fin shark	„	2·68	0·05
Carcharodon carcharias	Man-eater	„	36·13	33·55
Cetorhinus maximus (1)	Basking shark	„	47·73	39·31
	„ „	kidney	45·90	1·86
(2)	„ „	liver	32·75	24·52
(3)	„ „	„	35·32	28·82
Chimaera monstrosa	Common chimaera	„	74·80	62·83
Arctocephalus pusillus	Seal	„	29·41	0·50

H. K. Dean

Component Acids and Glycerides of Neat's Foot Oil. T. P. Hilditch and R. K. Shrivastava (*J. Soc. Chem. Ind.*, 1948, **67**, 139–142)—The mixed acids of a sample of neat's foot oil were subdivided into three groups by low temperature crystallisation, as follows—15·2 per cent. (iodine value 2·7) were obtained by crystallisation from 10 per cent. solution in ether at −40° C.; 57·1 per cent. (iodine value 85·2) were obtained from the remainder by crystallisation from 10 per cent. solution in acetone at −60° C.; the balance, 27·7 per cent., had iodine value 101·0. Each group was examined by the ester-fractionation method and the weight-percentages of the component acids were calculated as: myristic 0·7, palmitic 16·9, stearic 2·7, arachidic 0·1, tetradecenoic 1·2, hexadecenoic 9·4, oleic 64·4, octadecadienoic 2·3, octadecatrienoic 0·7, and unsaturated C_{20-22} acids 1·6. Constants of the oil were: saponification equiv. 286·3, iodine value 73·3, free fatty acids (as oleic) 0·7 per cent., unsaponifiable matter 0·3 per cent., and n_D^{40} 1·4610. The component glycerides were studied after partial separation by low-temperature crystallisation from acetone. The percentages of their constituents were approximately: palmitodiolein 35, hexadecenodiolein 23, polyethenoid-diolein 8, oleopalmitostearin 7, and probably not much more than 10 of triolein, with minor proportions of stearodiolein, palmito-polyethenoid-oleins, and hexadeceno-polyethenoid-oleins. The lubricant properties of the oil are probably not due to the small amount of triolein present, but may be connected with the specific *mixed* unsaturated glycerides present, *i.e.*, to hexadecenodiolein and perhaps also to the di-oleoglycerides in which the third acyl group is a polyethenoid member of the C_{18}, C_{20} (or C_{22}) series. The presence of fairly large proportions of hexadecenoic acid in neat's foot oil had not been previously noted.

E. B. Daw

Titration of Hyoscine, Hyoscyamine, and Atropine with Picric Acid in Chloroform: Separation and Fractionation of the Picrates. E. M. Trautner, O. E. Neufeld, and C. N. Rodwell (*Aust. Chem. Inst. J. Proc.*, 1948, **15**, 55–61)—Hyoscine, hyoscyamine, and atropine together with the related alkaloids and free bases associated with them in solanaceous plants can be titrated in chloroformic solution with picric acid in chloroform, dimethyl-amino-azo-benzene (butter-yellow) being used as an indicator. The colour change is from yellow to orange; it is sharp and similar to that of methyl orange in aqueous solution. During the titration, or shortly afterwards, hyoscine picrate is precipitated, and if trichloroethylene is added to the chloroform solution, the solubility of hyoscine picrate is sufficiently reduced for it to be quantitatively precipitated while hyoscyamine and atropine picrates remain in solution. The optimum mixture of chloroform and trichloroethylene is two to one and working with such a solvent, mixtures of hyoscine and hyoscyamine (or atropine) varying in proportions from 9 : 1 to 1 : 9 have been assayed with a maximum error of 10 per cent. of the amount of the constituent present in smaller amount.

Procedure—Shake 60 g. of finely-powdered leaf with 1000 ml. of industrial methylated spirit for several hours, filter the extract, and evaporate an aliquot to the consistency of a thick syrup. To the cold residue add 30 ml. of 3 per cent. phosphoric acid, allow the syrup to disintegrate to a well-defined precipitate, and filter. Shake the filtrate with successive small quantities of chloroform, wash the bulked chloroform extracts with a small amount of the 3 per cent. phosphoric acid solution, reject the chloroform, and add the acid washings to the bulk of the acid solution. Make the acid solution alkaline to phenolphthalein test paper by adding aqueous ammonia solution and extract with successive portions of chloroform. Mix the chloroformic extracts, wash with a few ml. of water, and evaporate to dryness in a current of air. At this stage make sure that the residue is entirely free from traces of chloroform or other solvents. Add 20 ml. of light petroleum (b.p. 90° to 110° C.), heat nearly to boiling, decant the supernatant liquid, and repeat with two successive portions of 10 ml. of light petroleum.

Mix the nearly water-white light petroleum fractions and extract quantitatively with successive portions of 3 per cent. phosphoric acid. Add aqueous ammonia solution to the bulked acid extracts until the mixture is alkaline to phenolphthalein test-paper and again extract with succesive quantities of chloroform. Adjust the volume of the final chloroform solution, either by dilution or by evaporation, to obtain an approximately 0·05 *N* solution of total alkaloids.

Titrate slowly with 0·05 *N* picric acid in dry chloroform to an orange-yellow colour, using dimethyl-amino-azo-benzene as indicator, and from the volume of 0·05 *N* picric acid used calculate the total alkaloids in the aliquot.

Add a volume of pure trichloroethylene equal to half the volume of the combined chloroformic solutions, allow to stand overnight, and filter. Wash the residue in the filter with chloroform, dry at 100° C., weigh, and calculate the proportion of hyoscine in the aliquot.

If the amount of total alkaloids in the sample is not even approximately known, extract the bulked light petroleum fractions with a known amount of standard acid, and titrate back with aqueous standard alkali to determine this concentration. Make the solution alkaline and extract the alkaloids as previously described. A. H. A. Abbott

Colorimetric Assay of Digitoxin. A. T. Warren, F. O. Howland, and L. W. Green (*J. Amer. Pharm. Assoc.*, 1948, **37**, 186–188)—The colorimetric method devised for the determination of digitoxin can be applied to tablets containing the drug. The procedure depends on the formation of a fugitive purple colour when digitoxin reacts with sodium β-naphthaquinone-4-sulphonate in alkaline solution, and addition of acetic acid at the time of maximum colour development to change the colour to yellow and to stabilise it.

Procedure—Prepare a chloroformic solution of the sample such that 1 ml. may be expected to contain approximately 20 µg. of digitoxin. Evaporate 10 ml. of the solution to dryness in a small flask under a gentle stream of air. Add 0·1 ml. of chloroform, swirl the flask to wet the residue, and then add 4 ml. of aldehyde-free ethyl alcohol and 0·5 ml. of 0·05 *N* sodium hydroxide. Place the flask in boiling water and exactly 1 min. later add, as rapidly as possible, 1 ml. of a reagent containing 0·024 per cent. of sodium β-naphthaquinone-4-sulphonate and 0·024 per cent. of sodium sulphite in water. Mix well and, exactly 1·5 min. after the addition of the reagent, add 0·5 ml. of 13 per cent. acetic acid in aldehyde-free alcohol. Remove the flask from the bath immediately, cool, and dilute to 25 ml. with alcohol. Measure the transmission of the solution within 2 hr. in a photoelectric colorimeter and make a blank determination on the reagents. Calculate the proportion of digitoxin in the sample by reference to a curve prepared by submitting aliquots of a standard solution of pure digitoxin in chloroform to the test procedure.

Procedure for tablets—Grind 20 tablets to fine powder and weigh a sample approximately equal to 10 tablets. Treat the powder with 20 ml. of boiling chloroform, filter the mixture, and wash the residue several times with warm chloroform. Combine the filtrate and washings, dilute to 50 ml. with chloroform, transfer 10 ml. of the solution to a small flask, and continue the assay as previously described. The above dilution is for tablets containing 0·1 mg. of digitoxin.

A. H. A. Abbott

Biochemical

Iodimetric Method of Estimation of Penicillin. A. M. Wild (*J. Soc. Chem. Ind.*, 1948, **67**, 90–92)—The method of Alicino (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 619; *cf. Analyst*, 1947, **72**, 68) has been studied and the following conclusions have been drawn.

(1) The degree of iodination is a linear function of temperature.

(2) The concentration of iodine has only a slight effect on absorption.

(3) The concentration of potassium iodide in the iodine solution used exerts a noticeable effect. That used by the author was 0·2 per cent.

(4) The *p*H should be between 6 and 7 and adjusted again to within that range before the final thiosulphate titration.

(5) The time of standing of each unhydrolysed sample in the presence of iodine should be carefully controlled according to its impurity content.

(6) Within the limits 0·1 to 0·33 *N*, neither the concentration nor the quantity of alkali has any effect on the subsequent titration. The reaction is complete within 15 min. and setting aside for more than 30 min. has no effect on the final result.

(7) Because of the different iodine absorption by impurities before and after alkali treatment, it is doubtful whether the method could be adapted to low-grade penicillin.

METHOD—*Procedure*—These conclusions lead to the following method of analysis being recommended.

Stock solutions—(1) 0·1 *N* Sodium thiosulphate containing also 0·2 g. of $Na_2CO_3.10H_2O$ per litre, and accurately standardised. (2) 0·1 *N* Iodine containing 2 per cent. of potassium iodide. (3) Aqueous phosphate buffer (I), *p*H 7·0; 2·835 g. of $NaH_2PO_4.2H_2O$; and 9·76 g. of $Na_2HPO_4.12H_2O$ per litre. (4) Aqueous phosphate buffer (II), *p*H 6·24; a mixture of 4 volumes of *M*/15 KH_2PO_4 and 1 volume of *M*/15 Na_2HPO_4. (5) 1 Per cent. starch solution in water.

Prepare 250 ml. of 0·01 *N* sodium thiosulphate by diluting the 0·1 *N* sodium thiosulphate with distilled water, free from carbon dioxide. Prepare 0·01 *N* iodine by pipetting 25 ml. of 0·1 *N* iodine into a 250-ml. graduated flask containing approximately 125 ml. of phosphate buffer (II), and diluting to volume with distilled water.

Procedure—Dissolve not less than 50 mg. of the sample in phosphate buffer (I) in a dry 250-ml. conical flask to give not more than 900 to 1000 units per ml. (Volume of solvent = *V*.) Pipette 5 ml. of the penicillin solution into each of two 4-oz. stoppered bottles *A* and *B*. Into *A* pipette 10 ml. of 0·01 *N* iodine, set aside in the dark for *exactly* 5 min., and titrate immediately with 0·01 *N* sodium thiosulphate, adding 2 ml. of starch solution.

Into *B* pipette 1 ml. of *N* sodium hydroxide and set aside for 30 min. Neutralise with 1 ml. of *N* hydrochloric acid. Add 10 ml. of 0·01 *N* iodine and set aside in the dark with another bottle (*C*) containing water and a thermometer. After 30 min., take the temperature (t°) in the bottle *C*, and immediately titrate the contents of *B* with 0·01 *N* sodium thiosulphate, adding 2 ml. of starch solution near the end-point.

Calculation—

Percentage of total penicillin =

$$\frac{(\text{Titre difference in } A \text{ and } B) \times V \times 100 \times \text{thiosulphate factor}}{\{2{\cdot}31 + 0{\cdot}015\ (\text{t} - 20)\}\ (\text{weight of sample}) \times 5}$$

For samples in which the relative proportion of penicillin G is high, a fair estimate of the potency is given by:—

$$\frac{(\text{Titre difference in } A \text{ and } B) \times V \times 1660 \times \text{thiosulphate factor})}{\{2{\cdot}31 + 0{\cdot}015\ (\text{t} - 20)\}\ (\text{weight of sample}) \times 5}$$

For precise results, the relative proportions of the penicillins must be known.

For maximum accuracy, the following points must be observed.

(1) Starch solution and 0·01 *N* sodium thiosulphate must be freshly prepared each day.

(2) A 10-ml. burette graduated in 0·02 ml. should be used.

(3) During the standing periods the temperatures of *A* and *B* should be within a few degrees of one another.

(4) Titrations of blank determinations should be done rapidly, especially when the penicillin is impure.

(5) The temperature in *B* while it is set aside should not vary more than ± 1° C. If fluctuations of more than ± 2° C. are encountered, thermostatic control at 25° C. is advised.

(6) The 5-ml. aliquot for assay must contain 4500 ± 500 units.

The reproducibility of the results obtained by this method is about ±1·5 per cent.

H. B. JOHNSON

New Colour Reactions and a New Method for the Photometric Determination of Vitamin B_1. C. S. Runti (*Intern. Z. Vitaminforschung*, 1948, **19**, 282)—This work originated as a study of the possibility of diazotising vitamin B_1. By reaction of a diazotised solution of aneurine hydrochloride with various amino compounds in presence of alkali, typical azo colours were obtained. To ascertain if these colours were due to a coupling of the diazo derivative of the vitamin with the amines or to the coupling of the diazo derivatives of the amines with the vitamin, the experiments were repeated after blocking the amino group of the vitamin by treatment with acetyl chloride. The colours were obtained as before and, similarly, the same colours were produced if the amino compounds were first diazotised, the excess of nitrous acid was removed by urea, the solution of vitamin added and the liquid made alkaline by adding potash. It concluded therefore that the vitamin is not itself diazotised under these conditions but reacts with the diazo derivatives of the amines employed. These included sulphonamides, *p*-aminobenzoic acid, and *p*-aminophenylarsonic acid. The reaction with 2(*p*-aminobenzene sulphonamido)pyridine was found to be particularly suitable for the photometric estimation of the vitamin. The concentration of the sodium nitrite solution employed has an important influence on the stability of the colours obtained. With the use of dilute (0·5 per cent.) solution these remain unchanged for a considerable time.

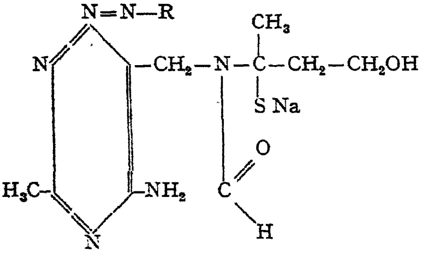
Estimation of vitamin B_1. Preparation of a standard curve—For this purpose use a Pulfrich step photometer and make a series of measurements with successive dilutions of a standard solution of aneurine hydrochloride containing, in 3 ml., 375 µg. of the vitamin. Measure 3 ml. of this solution or of its dilutions into a tared 25-ml. flask. Add 1 ml. of diluted hydrochloric acid (1 : 4) and 1 ml. of

0·5 per cent. sodium nitrite solution. Add 3 ml. of a saturated aqueous solution of 2(*p*-aminobenzene sulphonamido)pyridine, shake for 1 to 2 min. at room temperature and make alkaline by adding 1 ml. of a 33 per cent. solution of caustic potash. Allow to stand for 5 min., in which time the colour, at first orange, changes to pink. Dilute to the mark with water and after allowing to stand for 20 min., take readings in the photometer, 20 mm. cuvettes and the filter S.53 being used.

This procedure is specially adapted for the determination of the vitamin in pharmaceutical preparations. For biological material, a different standardisation would be required.

Of the other water-soluble vitamins, only ascorbic acid interferes with the results and this can be eliminated by oxidation with iodine.

From experiments with other substances containing a thiazole or pyrimidine nucleus the author concludes that the latter and not the former takes part in the reaction with the diazo compounds, and proposes the following general formula for the azo colours.



A. H. Bennett

Colorimetric Estimation of Pregnandiol in Urine. H. S. Guterman and M. S. Schroeder (*J. Lab. Clin. Med.*, 1948, **33**, 356–365)—Method—*Preparation of special reagent*—Dissolve 8 g. of sodium hydroxide pellets in 100 ml. of absolute methyl alcohol, allow to stand for a short time, and remove the precipitated carbonate by filtration. Determine the sodium hydroxide concentration of the solution by titration with 0·1 *N* sulphuric acid and adjust to a concentration of 2 per cent. with absolute methyl alcohol. Prepare the solution afresh each week.

Procedure—Boil 100 ml. of urine, 50 ml. of toluene, and 10 ml. of concentrated hydrochloric acid under a reflux condenser for 15 min., cool to room temperature, and transfer the mixture to a separating funnel. Allow to separate and, if an emulsion is formed, break it by addition of 1 or 2 drops of a detergent solution. Discard the lower (urine) layer and wash the toluene and any emulsion twice with 15 ml. of 0·1 *N* sodium hydroxide and then twice with 15 ml. of water. Transfer the washed toluene to a flask and boil off any dissolved water. When the toluene is boiling steadily add 10 ml. of 2 per cent. sodium hydroxide solution in absolute methyl alcohol and continue boiling until approximately half the original volume remains and a granular precipitate has formed. Filter hot, and if the filtrate is orange, pink or brown in colour, again add 10 ml. of 2 per cent. sodium hydroxide solution in absolute methyl alcohol and heat with more toluene until the volume has again been reduced by one half. When the filtrate is yellow, or yellowish-green, wash the residue in the filter with 15 ml. of hot toluene and evaporate the combined filtrate and washings to dryness. Remove the last traces of toluene by a stream of air.

Add 5 ml. of acetone to the residue, warm until dissolution is complete, slowly add 20 ml. of 0·1 *N* sodium hydroxide, and boil the mixture for 3 min. Cool, allow to stand at 5° C. for 1 hr., filter, and wash the residue with 15 ml. of water. Dissolve the residue in 10 ml. of hot absolute ethyl alcohol, evaporate the solution to dryness, and dissolve the residue in 10 ml. of concentrated sulphuric acid. Allow the solution to stand for 1 hr., dilute an aliquot to 5 ml. with concentrated sulphuric acid, and read the colour in a spectrophotometer at 430 mμ., concentrated sulphuric acid being used as a blank.

Prepare a standard solution of pregnandiol in absolute ethyl alcohol (100 μg. per ml.), evaporate aliquots containing 10 to 100 μg. of pregnandiol to dryness, dissolve the residues in 5-ml. quantities of concentrated sulphuric acid, allow to stand for 1 hr., and read the colours in a spectrophotometer. Plot the concentrations against the logarithms of the percentage absorptions and use the curve to determine the concentration of pregnandiol in the sample.

Recovery experiments indicate that the method has an average accuracy of 95 per cent., and androsterone was found not to interfere with the colour reaction.

A. H. A. Abbott

Delta-Tocopherol. I. Its Isolation from Soyabean Oil and its Properties. M. H. Stern, G. D. Robeson, L. Weisler, and J. G. Baxter (*J. Amer. Chem. Soc.*, 1947, **69**, 869–874)—α-, β-, and γ-tocopherols give substantially complete colour formation in 2 min. in a modified Emmerie and Engel assay method (see the next abstract) with only a small increase in colour intensity when the time is increased to 10 min. The mixture of tocopherols in the distillate from soyabean oil, however, showed an increase of about 10 per cent. The substance responsible for this has been separated and is a tocopherol, *viz.*, 8-methyltocol, differing from α-, β-, and γ-tocopherols in having only one methyl group in the aromatic ring of the chroman nucleus. It is the first monomethyltocol to be isolated from natural sources. It constitutes about 30 per cent. of the mixed tocopherols of soyabean oil (Weisler, Robeson, and Baxter, *infra*) and 5 per cent. of those in wheat germ oil (Kascher, unpubl.) and occurs in cottonseed and peanut oils (Weisler *et al.*, *infra*).

Properties—δ-Tocopherol is unique among the known tocopherols in giving with the ferric chloride and dipyridyl reagent a slow steady increase in colour intensity after the initial rapid oxidation is completed (see the next abstract). Of the tocopherols so far isolated it is the most active antioxidant for vitamin A and carotene *in vitro*, the order of decreasing activity being δ-, γ-, β-, α-tocopherol.

δ-Tocopherol reacts with diazo reagents and the reaction provides a method whereby it and also γ-tocopherol may be estimated in mixtures (see the next abstract).

Oxidation with 1·7 molecular proportions of gold chloride in ethanol for 45 min. according to the procedure of Karrer and Geiger (*Helv. Chim. Acta*, 1940, **23**, 455) yielded a reddish oil having the properties of a *p*-quinone ($E^{1\%}_{1\,\mathrm{cm.}}$ at 257 mμ = 222) that contained 12 per cent. of unchanged δ-tocopherol. When δ-tocopherol (0·055 g.) and silver nitrate (0·15 g.) were heated with 1 ml. of ethanol at 50° C. for 1 hr. and the product was extracted with ether, a reddish oil having the ultra-violet light absorption properties of an *o*-quinone ($E^{1\%}_{1\,\mathrm{cm.}}$ at 435 mμ = 15·2) and containing 6·5 per cent. of unchanged tocopherol was obtained. When δ-tocopherol (0·03 g.) in 10 ml. of ethanol is treated slowly with 22 ml. of nitric acid and, after shaking, is allowed to stand for 2·5 hr. at room temperature the orange-yellow pigment formed has an extinction coefficient at 373 mμ. of 59. Oxidation of α-, β-, and γ-tocopherols with nitric acid yields *o*-quinones. The different behaviour of δ-tocopherol with nitric acid may possibly provide a method for its determination in mixtures with other tocopherols. Possibly the mixture might be oxidised with nitric acid and the δ-tocopherol determined spectrophotometrically by measuring the intensity of the absorption at 373 mμ.

The experimental determination of the structure of δ-tocopherol is described. A. O. Jones

Delta-Tocopherol. Assay of Total Tocopherols in Mixtures Containing Delta-Tocopherol. M. H. Stern and J. G. Baxter (*Anal. Chem.*, 1947, **19**, 902–905)—Delta-tocopherol is distinguished from α-, β-, and γ-tocopherols by the fact that it gives more colour on a molecular basis with the ferric chloride and α,α′-dipyridyl reagent of Emmerie and Engel (*Rec. Trav. Chim.*, 1938, **57**, 1351; *Analyst*, 1939, **64**, 216), so that its presence in a mixture of tocopherols results in assay values that are too high.

The reagent of Emmerie and Engel has been used in acetic acid solution and in ethanol solution. The assay of mixtures of tocopherols by means of the reagent in acetic acid is unsatisfactory because the intensity of colour produced with the individual tocopherols is not a linear function of the concentration, and lack of linearity makes it difficult to standardise a method which is to be applied to mixtures in which the proportion of the various tocopherols is unknown. Also the rate of oxidation of the individual tocopherols by the acetic acid reagent varied, so that a time could not be selected in which all the tocopherols gave about the same colour intensity, and it would thus be difficult to use the reagent for assaying mixtures of tocopherols except where the composition of the constituents was known and it was possible to calibrate the method with pure tocopherols having the same composition.

When the reagent is used in ethanol solution the rate of oxidation of α-, β-, and γ-tocopherols is rapid and the intensity of the colour produced is substantially the same when allowance is made for differences in the molecular weights. Colour formation with α-, β-, and γ-tocopherols is complete in 2 min. with only a slight increase when the reaction time is extended to 10 min. With δ-tocopherol, however, extension of the time of reaction causes an increase of 30 per cent. in the colour, and thus time becomes a critical factor in mixtures containing this tocopherol. For mixtures of unknown composition the best reaction time is 2·5 min., since colour formation for three of the tocopherols is then complete and the colour intensities for all four are most nearly the same. If this time is taken as the basis of an assay procedure for mixtures containing δ-tocopherol, the assay may be standardised either with pure natural α-tocopherol or preferably with a 50 to 50 mixture of α- and γ-tocopherols.

Procedure—Separate solutions of ferric chloride (0·1 g. of $FeCl_3.6H_2O$ per 100 ml.) and α,α′-dipyridyl (0·25 g. per 100 ml.) in purified absolute ethanol are prepared and stored in amber glass bottles, preferably painted black, fresh solutions being made every 2 weeks. To a 1-ml. aliquot of the sample containing 50 to 150 μg. of tocopherol in purified ethanol (for concentrates) or light petroleum (for low potency preparations containing glycerides) in a 2-oz. glass-stoppered bottle painted black add, in order, 1 ml. of the dipyridyl reagent, 1 ml. of ferric chloride reagent, and 22 ml. of purified ethanol, the last from a 50-ml. burette. When 17 ml. have been added, start a clock with a seconds hand while the remainder is being run in, insert the stopper, swirl the mixture gently and set it aside for 2·5 min. A blank liquid previously prepared (1 ml. of ethanol, 1 ml. of each reagent, then 22 ml. of ethanol) is used to adjust the galvanometer of an Evelyn colorimeter to 100 with a No. 520 filter. About 10 sec. before the completion of the 2 5 min. period pour the test solution into the tube in the colorimeter, cover it with a black cap, and take the galvanometer reading at 2·5 min. Determine the tocopherol content by means of a curve relating the *L* value (2 − log *G*) to the concentration, which has been prepared either with pure natural α-tocopherol or with a 50 to 50 mixture of natural α- and γ-tocopherols. With galvanometer readings of 25 to 70, *L* is a linear function of the concentration. The absolute ethanol must be purified by distillation with potassium permanganate and potassium hydroxide through a Vigreux column, and the light petroleum (Skellysolve B, b.p. 64° to 68° C.) by washing with concentrated sulphuric acid, and then with dilute sodium hydroxide and distilling. Excessive exposure of either the sample solution or the blank solution to light must be avoided.

Improved procedure for assay of soyabean oil—A sample of crude soyabean oil was "degummed" by stirring at 40° C. with 2 per cent. by weight of water. The sludge was allowed to separate overnight and the oil was filtered through a bed of sodium sulphate to remove traces of water. To a 10-ml. aliquot of purified light petroleum containing about 0·1 g. of the oil in a conical glass-stoppered centrifuge tube, 2 ml. of 85 per cent. (w/w) sulphuric acid were added and the tube was inverted five times and centrifuged. The supernatant liquid was decanted into a clean tube, shaken with 5 ml. of 1 per cent. potassium hydroxide solution, and again centrifuged. A 3-ml. aliquot of the liquid, containing about 60 µg. of tocopherol, was placed in a reaction bottle and the solvent was removed in a stream of nitrogen. To the residue were added light petroleum (1 ml.), dipyridyl reagent (1 ml.), ferric chloride reagent (1 ml.) and ethanol (22 ml.). The value of L was determined by the procedure of Baxter *et al.* (*Biol. Symposia*, 1947, **12**, 484), which is substantially that already described but with a 2-min. reaction period. Reference to a calibration curve prepared with a 50 to 50 mixture of α- and γ-tocopherols gave the partly corrected value of 0·176 per cent. for the total tocopherol content of the water-refined oil. A correction was made for the inhibition of colour formation that occurs with vegetable oils and with some mineral oils by the method of Kaunitz and Beaver (*J. Biol. Chem.*, 1944, **156**, 653). A 3-ml. aliquot containing about 60 µg. of pure γ-tocopherol was treated with the dipyridyl reagent, the ferric chloride reagent and ethanol in the usual way and the L value was determined after a reaction time of 2 min. Another aliquot was evaporated to dryness in a stream of nitrogen and a 3-ml. aliquot of the light petroleum sample solution (after purification with sulphuric acid and potassium hydroxide) containing about 60 µg. of tocopherols was added to it and the L value determined. If L_t, L_s, and L_c represent the values of L for the pure tocopherol, the sample solution and the combination of both respectively, the percentage recovery is $100\ L_t/L_c - L_s$. The value of 0·176 per cent. was thus corrected for the inhibition and the value 0·190 per cent. was found for the soyabean oil. Further work suggests that this assay procedure would be improved by the use of the 2·5-min. reaction period in the assay and by the use of a 70 to 30 mixture of γ- and δ-tocopherols in the inhibition determination.

The cause of the inhibition of colour formation occurring with many vegetable oils is not known with certainty. In assaying preparations of low potency a correction for inhibition should be applied and in its determination a mixture of pure tocopherols having approximately the same composition as those in the sample should be used.

A. O. Jones

Delta-Tocopherol. Assay of Individual Tocopherols in Mixtures Containing Delta-Tocopherol. L. Weisler, C. D. Robeson, and J. G. Baxter (*Anal. Chem.*, 1947, 19, 906–909)—When γ- and α-tocopherols were believed to be the only commonly occurring members of the vitamin E complex, their determination in mixtures was relatively simple and procedures based on the determination of γ-tocopherol by selective oxidation with silver nitrate or nitric acid or by coupling with diazotised *p*-nitraniline were developed, α-tocopherol being then found by difference from the total tocopherol content as measured by the Emmerie and Engel method (*Rec. Trav. Chim.*, 1938, **57**, 1351; Analyst, 1939, **64**, 216). Another method was the direct determination of α-tocopherol by selective oxidation with ferric chloride (Hove and Hove, *J. Biol. Chem.*, 1944, **156**, 601). The presence of δ-tocopherol causes considerable error in these methods, since it behaves like γ-tocopherol, giving a colour with silver nitrate and with nitric acid.

The method now presented for determining the individual tocopherols in mixtures of α-, γ-, and δ-tocopherols depends upon coupling duplicate samples with diazotised *o*-dianisidine in alkaline solution. Both γ- and δ-tocopherols have unsubstituted positions in the aromatic ring of the chroman nucleus and couple with the reagent to give red pigments absorbing at 510 mµ. The relative intensity of colour formation for the two tocopherols at different degrees of alkalinity, however, is different, and this difference provides a basis for determining each in mixtures. α-Tocopherol has no unsubstituted position and hence does not couple, but it can be measured by difference from the total tocopherol content determined by the modified Emmerie and Engel procedure of Stern and Baxter (see the preceding abstract).

To prepare *o*-dianisidine dihydrochloride add to 100 g. of crude powdered dianisidine (m.p. 134° to 136° C.) 140 ml. of water and 6 ml. of concentrated hydrochloric acid, stir well, boil gently until dissolution is complete, add 0·5 g. of stannous chloride, boil for 5 min., add 2 g. of decolorising carbon, boil gently for a further 5 min., and filter rapidly by suction through Celite. To the hot filtrate add 50 ml. of concentrated hydrochloric acid, cool in an ice-bath and collect the crystals by suction, washing three times with ethanol and once with ether, and dry the crystals at 40° C. for 1 hr. (m.p. 283° C. with decomposition).

To prepare the diazotised reagent add 6 ml. of concentrated hydrochloric acid to 0·5 g. of *o*-dianisidine dihydrochloride dissolved in 60 ml. of water and then add 12 ml. of 5 per cent. aqueous sodium nitrite solution. Mix thoroughly and, after 5 min., add 12 ml. of 5 per cent. aqueous urea solution. Set the thoroughly mixed reagent aside for 24 hr. at room temperature before using. It is stable for about 10 days.

Procedure—Two coupling reactions are necessary on duplicate samples of the preparation. Dilute a sample containing 50 to 100 µg. of γ- and δ-tocopherols to 3 ml. with absolute ethanol in a 50-ml. glass-stoppered cylinder. Add 7·5 ml. of a 2 per cent. aqueous solution of sodium carbonate monohydrate and 1 ml. of diazotised *o*-anisidine solution, shake the mixture, allow it to stand at room temperature for 5 min., then add 0·5 g. of sodium sulphate and 12 ml. of light petroleum (Skellysolve H). Shake the mixture vigorously three times, allowing the phases to separate completely after each shaking. Prepare a blank liquid by the same procedure, omitting the tocopherols. The small interfacial precipitate that forms does not affect the determination. Transfer the mixture to a separating funnel and discard the lower phase. Dry the light petroleum layer with sodium sulphate and determine its L value ($2 - \log G$) in an Evelyn colorimeter with a No. 250 filter and with the instrument set at 100 for the blank liquid.

For the second coupling, dilute a sample containing 50 to 200 µg. of γ- and δ-tocopherols to

4 ml. with absolute ethanol in a 50-ml. glass-stoppered cylinder, add 2 ml. of a 2 per cent. solution of potassium hydroxide in absolute ethanol and 0·3 ml. of diazotised *o*-dianisidine solution. Shake the mixture, allow it to stand for 2 min., and then add 8 ml. of water, 12 ml. of light petroleum, and 0·5 g. of sodium sulphate. Extract the dye in the manner described for the coupling reaction in sodium carbonate solution, preparing a blank solution in the same manner.

To prepare calibration curves couple the two pure tocopherols at the two alkalinities and plot the *L* values against the concentrations. The high intensity produced by coupling δ-tocopherol in sodium carbonate solution contrasts with the lower intensity produced by coupling in potassium hydroxide solution. The curves for the dye from γ-tocopherol are intermediate. Finally determine the total tocopherols by the method described in the preceding abstract.

The use of these differences in colour formation to assay γ- and δ-tocopherols is based on two equations relating the intensity of colour formed in the two alkaline solutions to the percentage of individual tocopherols present.

$$L^{\mathrm{S}}_{\mathrm{C}} = (\%\ \gamma)L^{\gamma}_{\mathrm{C}}/100 + (\%\ \delta)L^{\delta}_{\mathrm{C}}/100$$

$$L^{\mathrm{S}}_{\mathrm{H}} = (\%\ \gamma)L^{\gamma}_{\mathrm{H}}/100 + (\%\ \delta)L^{\delta}_{\mathrm{H}}/100$$

where L^{γ}_{C}, L^{δ}_{C}, L^{γ}_{H}, and L^{δ}_{H} are constants representing the *L* values of pure tocopherols coupled in sodium carbonate and potassium hydroxide solutions and $L^{\mathrm{S}}_{\mathrm{C}}$ and $L^{\mathrm{S}}_{\mathrm{H}}$ represent the observed *L* values for the total tocopherols in the sample being assayed. The *L* value is $(2 - \log G)/1{\cdot}9\ C$, where *G* is the galvanometer reading and *C* is the number of grams of total tocopherols per 100 ml. in the standard volume used (12 ml.). By substituting the experimentally determined *L* values and solving the equations, the percentages of γ- and δ-tocopherols are calculated.

The accuracy of the diazo method can often be increased by a preliminary adsorption to separate α- and γ-tocopherols from δ-tocopherol. Only a single calibration curve, preferably prepared by coupling γ-tocopherol in potassium hydroxide solution is then needed. The firmness of adsorption of these three tocopherols on zinc carbonate increases in the order α, γ, δ. By passing a light petroleum solution of mixed tocopherols through a column of zinc carbonate and Celite under controlled conditions, it is possible to collect the α-tocopherol and a portion of the γ-tocopherol in the filtrate, leaving the δ-tocopherol and certain pigments on the column. Application of the diazo reaction to the tocopherols recovered from the filtrate permits calculation of the percentage of the α-compound in the tocopherols of the original sample, since the total tocopherol content of the latter can be determined and the weight of sample adsorbed is known.

A satisfactory column (2·5 × 60 cm.) can be prepared by inserting 50 g. of a 70 : 30 mixture of zinc carbonate and Celite in four portions in the tube, suction being applied from a water pump through a filter flask after each addition. Since the adsorption method depends upon the separation of the α-tocopherol from such interfering reducing compounds as δ-tocopherol, the ratio of tocopherol concentrate to adsorbent which will allow all the α-tocopherol and a portion of the γ-tocopherol but no δ-tocopherol to pass through the column must first be determined. The diazo method is used to establish absence of δ-tocopherol from the filtrate. A 2-g. sample is satisfactory for concentrates having a potency of approximately 30 per cent. of mixed tocopherols. For higher concentrates (60 to 80 per cent.) a 1-g. sample is preferable. First wash the column with 125 ml. of light petroleum, add the sample in 25 ml. of light petroleum and rinse it into the column with another 25-ml. portion of solvent. Draw the solution through the column by gentle suction and wash with 300 ml. of light petroleum. The top of the column must remain covered with solution or solvent at all times after the initial wetting. Follow the course of the washing operation by means of ultra-violet light.

Clean, sharp bands are observed if the packing is satisfactory, and the α-tocopherol is associated with three blue fluorescent bands that pass down the column first and into the filtrate. Evaporate the filtrate on the steam-bath under a stream of nitrogen and weigh the residue. Then assay it for total tocopherols as described in the preceding abstract and for γ-tocopherol by the diazo reaction. The percentage of α-tocopherol in the original sample is then readily calculated. A. O. JONES

Determination of Individual Tocopherols. M. Kofler (*Helv. Chim. Acta*, 1947, **30**, 1053–1070)—δ-Tocopherol (8-methyltocol), recently isolated from soyabean oil, resembles α-tocopherol (5 : 7 : 8-trimethyltocol) in biological activity, whereas β-tocopherol (5 : 8-dimethyltocol) and especially γ-tocopherol (7 : 8-dimethyltocol) exhibit less activity. Synthetic 5 : 7-dimethyltocol (not yet found in nature) and synthetic 8-monomethyltocol (possibly a stereo-isomer of the natural product) are biologically inactive. Methods for the determination of individual tocopherols are therefore required.

OXIDATION METHODS—In methods depending upon oxidation to tocopheryl *p*-quinones the consumption of oxidising agent is directly or indirectly measured. *Ceric sulphate method*—Dissolve about 10 mg. of the tocopherol in 10 ml. of alcohol, add 2 drops of diphenylamine indicator solution, and titrate with 0·01 *N* ceric sulphate until the colour changes to violet. If the colour change is indistinct use leuco-malachite green as external indicator. Each millilitre of the ceric sulphate solution ≡ 2·15 mg. of α-tocopherol, 2·08 mg. of dimethyltocol, and 2·01 mg. of δ-tocopherol. In the *ferric chloride - dipyridyl method* of Emmerie and Engel (*Rec. Trav. Chim.*, 1938, **57**, 1351; *Analyst*, 1939, **64**, 216, 446) remove the solvent from the tocopherol extract and treat the residue with 15 ml. of the reagent. After 10 min. with α- or β-tocopherol and 30 min. with γ- or δ-tocopherol, measure the extinction in a Pulfrich photometer with filter S.50 and a cell thickness of 5 cm. against the reagent in a similar cell. To prepare the reagent add 100 parts of alcohol to 1 part of an aqueous 20 per cent. solution of ferric chloride hexahydrate, mix this solution with an equal volume of a 0·05 per cent. solution of α,α′-dipyridyl in alcohol and store in the dark. Determine the concentration of the α- or β-tocopherol by means of a standard curve. In the determination of β-tocopherol the α-curve may be used if the final result, in accordance with the difference in the molecular weights, is reduced by 3 per cent. For the determination of tocopherol in blood plasma shake 5 to 10 ml. with light petroleum and adsorb the tocopherol chromatographically on weakly active aluminium oxide, subsequently eluting the tocopherol with benzene. This purification is particularly necessary with bovine plasma to separate carotenoids. With human plasma, in which only the α-compound occurs in measurable amount, and with rat plasma, the results from purified and unpurified samples differ by only 5 to 20 per cent. Treat the benzene eluate after removal of the solvent as already described, and determine the concentration from the extinction curve.

The Furter - Meyer reaction—(*Helv. Chim. Acta*, **1939**, **32**, 240; *Analyst*, 1939, **64**, 217). All four tocopherols form reddish-brown oxidation products when treated with nitric acid in alcohol, but the optimum reaction period is different for the individual compounds. To 10 ml. of the alcohol solution add 2 ml. of concentrated nitric acid, heat to the boiling point, place the mixture in boiling water for 5 min., cool, and dilute with alcohol to 20 ml. Determine the extinction in a Pulfrich photometer against alcohol. α-Tocopherol does not give this reaction in chloroform solution. For the determination of β-, γ-, or δ-tocopherol (also in presence of α-tocopherol) by oxidation in chloroform solution, treat 10 ml. of the solution in a 25-ml. stoppered cylinder with 1 ml. of concentrated nitric acid and shake for 15 sec. After a few more sec., and before nitrogen peroxide vapour is evolved, withdraw the chloroform layer, filter it into a thin-walled test-tube, place this for a few seconds in warm water to dispel turbidity during the measurement and measure the colour in a Pulfrich photometer with filter S.50 and a cell thickness of 2 cm. against chloroform as blank liquid.

FLUORIMETRIC METHOD—According to Smith *et al.* (*J. Amer. Chem. Soc.*, 1939, **61**, 2424) *o*-quinones exist in the red compound formed by oxidation of the tocopherols with nitric acid. The evidence for this is that these compounds condense with *o*-phenylenediamine to form phenazine derivatives, and, on the basis of this reaction, a fluorimetric method has been developed. With α-tocopherol, the oxidation must be made in alcohol; with β- and γ-tocopherols, it may be made in alcohol or chloroform. For the determination in alcohol the procedure is as previously described (*Helv. Chim. Acta*, 1943, **26**, 2166; *Analyst*, 1944, **69**, 156). The determination is best made in a fluorimeter, otherwise comparison solutions are necessary. If equal amounts of α- and γ-tocopherol are converted into their phenazine derivatives, the product from γ-tocopherol exhibits only about half the fluorescence of the derivative of the α-compound and also the fluorescence is greener. The method is not so suitable for β- and δ-tocopherol nor for 5 : 7-dimethyltocol, but it may be used for these compounds if the phenazine derivative is purified on a weakly active aluminium oxide column. γ-Tocopherol can be determined fluorimetrically in presence of α-tocopherol if the oxidation is made in chloroform solution. Treat 10 ml. in a stoppered graduated cylinder with 1 ml. of concentrated nitric acid, shake for 2 min., add 20 ml. of low-boiling petroleum spirit and about 20 ml. of water, shake, remove the aqueous layer, wash with water, and then with strong brine, and remove the solvent. Condense the residue with *o*-phenylenediamine as usual in glacial acetic acid. This method also is less suitable for β- and δ-tocopherol. The glacial acetic used must be purified with animal charcoal since even the best commercial grades contain an inhibitor of the condensation. The brown colour that the diamine solution acquires in air does not interfere.

THE DIAZOTISATION METHOD—This method is applicable only to γ- and δ-tocopherols. To prepare the diazonium salt solution dissolve 0·7 g. of *p*-nitraniline in 20 ml. of diluted hydrochloric acid (1 + 1), place the solution in ice and, immediately before use, mix 1 part of this solution with 1 part of a cooled 1 per cent. sodium nitrite solution. Treat 15 ml. of an alcoholic solution of γ- or δ-tocopherol and, at the same time, 15 ml. of alcohol, each with 1 ml. of the diazonium salt solution. After 60 min. measure the extinction in a Pulfrich photometer with filter S.53 with a cell-thickness of 5 cm., keeping the solutions in the dark until the measurement is made. If the coloured solution is turbid, add, after the 60-min. period, 1 ml. of water, extract with about 15 ml. of light petroleum (b.p. 80° to 100° C.), wash the extract with water, filter, and adjust the volume to 20 ml. Measure the colour as before against the solvent as blank liquid. If the petroleum extract contains other coloured substances, chromatographic separation may be tried with a short column of weakly active aluminium oxide, eluting with a mixture of light petroleum and benzene. The composition of the eluate depends upon the activity of the oxide (*infra*).

MIXTURES OF TOCOPHEROLS—To a mixture of another tocopherol with α-tocopherol apply the nitric acid method in chloroform solution. At the same time the colour will indicate which tocopherol is present, a red-violet indicating β-tocopherol and a red-orange, γ- or δ-tocopherol. To estimate γ- and δ-tocopherols in presence of α- and β-tocopherols use the diazo method. To determine β-tocopherol in presence of α-tocopherol use the nitric acid method. If γ- or δ-tocopherol is present, the β-compound must be determined by difference, the γ- and δ-compounds being determined by the diazo reaction and the β-, γ- and δ-compounds by the nitric acid reaction in chloroform. α-Tocopherol can be separated chromatographically from γ- and δ-tocopherols. In presence of β-tocopherol it can either be determined fluorimetrically (when practically only the α-compound is measured) or the β-tocopherol can be determined by the nitric acid reaction in chloroform and then the α- and β-compounds by the ferric chloride reaction or the nitric acid reaction in alcohol. If, at the same time, γ- or δ-tocopherol is present these are separated from α-tocopherol chromatographically, the α-compound with a portion of the β-compound being found in the benzene eluate.

To prepare the aluminium oxide for the chromatographic separations treat 4 kg. of the oxide with 2 litres of concentrated hydrochloric acid in which 200 g. of stannous chloride have been dissolved. Add 2 litres of water and shake the mixture from time to time. After 3 days wash the treated oxide with distilled water by decantation and finally on a Buchner funnel until the washings are no longer acid to methyl red. Finally wash the oxide with acetone and dry it in air. Ignite a portion of the oxide and re-mix it with the un-ignited portion. Determine the correct degree of activity for the oxide with α- and γ-tocopherol, adding so much ignited to un-ignited oxide that α-tocopherol but not γ-tocopherol is quantitatively eluted with methanol.

The theoretical bases of the methods described are discussed and examples are given of their application to the determination of individual tocopherols in wheat germ oil and cottonseed oil.

A. O. JONES

Bacteriological

A Strain of *Lactobacillus plantarum* suitable for use in the Estimation of Antiseptic Power [of Hops, Hopped Worts, and Beers]. D. Kulka and T. K. Walker (*J. Inst. Brew.*, 1948, **54**, 130–132)—This lactic acid bacterium was substituted for Massol's bacterium, a strain of *Lactobacillus bulgaricus* isolated from St. Ivel cheese, which had lost its acid-forming capacity.

It was isolated by Walker and Hastings [also from St. Ivel cheese] and proved by them to be very trustworthy in the study of hop antiseptic by the log-phase methods during the period 1933–40. In 1946, cultures that had been grown in liquid wort, neutralised with calcium carbonate and sealed, were found to behave exactly like the original cultures 14 years before in their resistance to phenol and humulone. Detailed examination of the organism was not then made, but has been carried out by the present authors, who now give a full description and show that it is not a strain of *Lactobacillus bulgaricus* but corresponds very closely to the *Lactobacillus plantarum* strain: Reading 4125 of the collection of the National Institute for Research in Dairying.

It is a straight-sided, Gram-positive bacterium of variable length, non-motile and without spores, and a facultative anaerobe. It gives no catalase reaction, gives a positive Voges-Proskauer reaction, and acid production is given with maltose, lactose, sucrose, L-arabinose, D-xylose, glucose, fructose, raffinose, and salicin. It does not, like *L. casei*, peptonise sour milk at 14 days at 25° C.

D. R. WOOD

Agricultural

Quantitative Estimation of Hemicelluloses by Direct Isolation. H. D. Weihe and M. Phillips (*J. Agric. Res.*, 1947, **74**, 77–85)—In the method of Preece (*Biochem. J.*, 1931, **25**, 1304) the sample (20 to 25 g.) is extracted first with a hot 0·5 per cent. ammonium oxalate solution, then with a 1 per cent. boiling sodium hydroxide solution in 50 per cent. ethanol, and finally with 50 per cent. ethanol. The residue is then repeatedly extracted at room temperature with a 4 per cent. aqueous sodium hydroxide solution until a portion of the extract no longer gives a precipitate when neutralised with acetic acid and diluted with an equal volume of acetone. The combined alkaline extracts are first acidified with glacial acetic acid and then treated with an equal volume of acetone. The precipitate is collected and dried *in vacuo*, and the weight of the moisture-free and ash-free product is determined and reported as free hemicellulose. The material remaining from the extraction with the alkali solution is then extracted with 4 per cent. boiling sodium hydroxide solution, the product is isolated in the manner described, and the result is reported as combined hemicellulose.

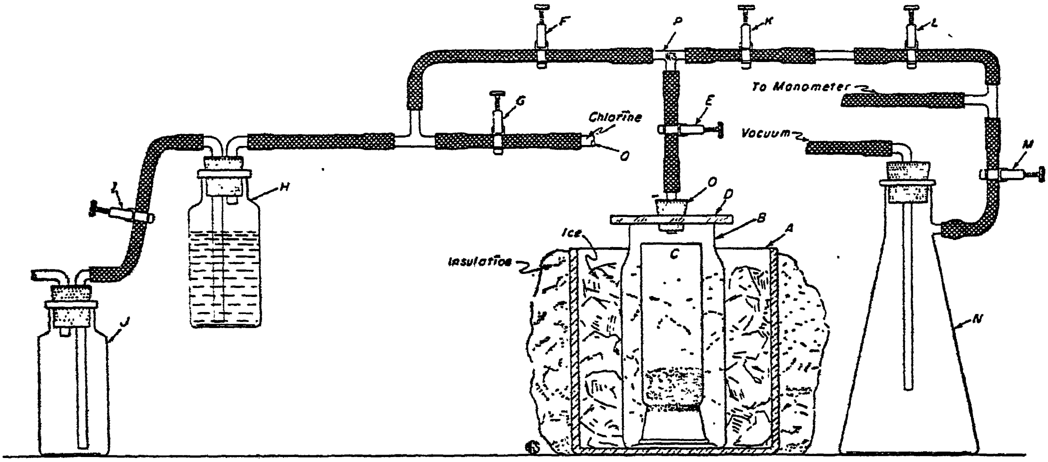
In this procedure the free and combined hemicellulose fractions are both contaminated with lignin and considerable degradation of the combined hemicellulose is likely to occur when it is extracted with boiling sodium hydroxide solution.

The method now presented consists essentially of the isolation of the holocellulose from a sample that has previously been freed from alcohol-benzene soluble extractives and pectic substances, and isolation of hemicellulose from the holocellulose. The residual material is weighed to determine the total amount of material extractable with dilute sodium hydroxide solution.

For determining pectic substances and holocellulose the apparatus recommended consists of a sintered-glass crucible (5½ in. × 1¾ in.) with a disc of 80- to 100-mesh. The end below the disc is rimmed to take a rubber stopper carrying a glass tube bent upwards parallel to the length of the crucible and connected with suction apparatus for removing solvent from the crucible.

To determine the alcohol - benzene (1 + 2) extractives place a 5-g. sample of the plant material in the crucible and extract it in a Soxhlet extractor for 30 hr. Remove as much as possible of the solvent by suction, dry the crucible *in vacuo* at 60° C., and weigh. For the extraction of pectic and other substances soluble in 0·5 per cent. aqueous ammonium oxalate solution, attach the rubber stopper and suction tube, nearly fill the crucible with 0·5 per cent. ammonium oxalate solution at 85° C., and place the assembly in a beaker of water maintained at that temperature for 1 to 1·5 hr., stirring the contents occasionally. Remove the solvent by suction and continue the digestion repeatedly with fresh solvent until a portion of the extract, when treated with four times its volume of ethanol previously acidified with hydrochloric acid, gives no precipitate. Finally, wash the residue with water until free from ammonium oxalate, then with 96 per cent. ethanol, dry *in vacuo* at 60° C., and weigh.

The procedure for determining the holocellulose in the residue is a modification of that of Ritter *et al.* (*Paper Trade J.*, 1941, **113**, 83, Tappi sect., 143; 1937, **105**, 127, Tappi sect., 277). The apparatus for chlorination, shown in the figure, consists of a glass jar *B* with a tightly fitting, ground-glass cover *D* carrying a rubber stopper *O*. The glass tube passing through *O* is connected by means of rubber tubing provided with a Hofmann clamp *E* to the T-tube *P*, which, in turn, is connected in the manner shown to the bottles *H* and *J* and to the litre suction flask *N* containing diluted sodium hydroxide solution for the absorption of chlorine. Clamps are provided at *F*, *G*, *I*, *K*, *L*, and *M*. *H* is almost filled with saturated sodium chloride solution. Chlorine from a cylinder enters the system at *Q*. By closing the clamp *F* and opening clamps *G* and *I*, chlorine from *Q* can be led into *H* and the salt solution driven over into *J*. *B* is placed in an insulated jar *A* containing crushed ice. The sample to be chlorinated is placed in the sintered-glass crucible *C*.



Moisten the sample in *C*, which has been successively extracted with alcohol-benzene mixture and with 0·5 per cent. ammonium oxalate solution (*supra*) with about 12 ml. of water and place the crucible in the chlorination chamber *B*. Place *B* in *A* and fill the latter with crushed ice. Place the cover on *B* and replace *O* with a thermometer in a suitable stopper and with its bulb within an inch of the sample in *C*. When the temperature in *C* is 15° C. or less, remove the thermometer and insert *O*. With *F* closed and *E*, *K*, and *L* open, evacuate the system by opening *M*. By means of a manometer ensure that the system is gas-tight. and, with *K*, *L*, and *M* closed and *G* and *I* open, pass chlorine from a cylinder through a water wash-bottle into the system at *Q* until about one-half of the salt solution in *H* is forced into *J*. Open *F* slowly and increase and adjust the flow of chlorine so that the level of the liquid in *H* remains almost constant. When the pressure in *B* is approximately atmospheric, allow *H* to fill with chlorine, then close the clamp *G* and simultaneously stop the chlorine supply. The level of the liquid in *H* will rise as chlorine is absorbed by the sample. When absorption has ceased, close *F*, remove *C* from the chlorination chamber, and thoroughly mix the contents with 95 per cent. ethanol. After 2 min., draw off the solution in *C* with the aid of a suction flask and repeat the washing. Add to *C* a 3 per cent solution of ethanolamine in 95 per cent. ethanol and stir. Draw off the liquid and repeat the washing. Wash again twice with ethanol and once with ether and, finally, free the sample from solvent by means of the suction pump. Moisten the sample with 12 ml. of water and repeat the process of alternate chlorination and extraction, as described, until the chlorinated sample no longer gives a colour with ethanolamine solution. Dry the crucible and its contents *in vacuo* at 60° C., weigh, and calculate the amount of holocellulose in the sample.

To isolate the hemicelluloses from holocellulose add to 1 g. of holocellulose in a 200-ml. Erlenmeyer flask 100 ml. of a 4 per cent. aqueous sodium hydroxide solution, stopper the flask and digest the mixture at room temperature (25° to 30° C.) for 2 hr. with occasional stirring. Filter the mixture through cloth in a small Hirsch funnel, wash the residue with 15 ml. of water, and add the washings to the main filtrate. Repeat the digestion in the same manner three more times. To the combined filtrates add 3 volumes of ethanol, make the liquid slightly acid with acetic acid, and allow it to stand overnight at room temperature. Draw off the supernatant liquid, collect the hemicellulose material in a sintered-glass crucible of fine porosity, washing with 100 ml. of a mixture of 25 ml. of water, 1 ml. of glacial acetic acid, and 75 ml. of 95 per cent. ethanol. Wash successively with 95 per cent. ethanol, absolute ethanol, and ether. Finally, dry *in vacuo* and weigh the hemicelluloses.

In this work hemicelluloses are defined as carbohydrate complexes that are extracted from holocellulose by diluted aqueous sodium hydroxide solution at room temperature and that are precipitated from the alkaline extract by excess of ethanol. This definition is no more arbitrary than any other that might be proposed. A. O. JONES

Organic

Automatic Micro-determination of Carbon and Hydrogen. R. O. Clark and G. H. Stillson (*Anal. Chem.*, 1947, **19**, 423–426)—Combustion cycles for the analysis of a wide range of samples were determined, a semi-automatic combustion unit being used.

METHOD—*Apparatus*—The vaporisation furnace is 6·35 cm. long and contains six heating elements, each consisting of a length of Alundum thermocouple tubing (4·25 mm. outside diameter) wound with No. 30 platinum wire to give a total resistance of 5·5 ohms with the windings connected in series. The windings are spaced so that the end of the furnace nearer the combustion furnace operates at a temperature 50° C. higher than the other. The temperatures of the vaporisation and combustion furnaces, the pre-burner and heating mortar are all read, through a selector switch, on a single millivoltmeter calibrated in degrees centigrade. The vaporisation furnace is advanced by means of a threaded brass half-bearing and Monel-metal screw, both having ⅜-in. thread, 12 threads to the inch. This permits the furnace to be moved manually to any position by lifting the bearing from the screw drive, which is driven by a 100 r.p.m. uni-directional motor. Power is transmitted by a 30 to 1 worm and gear combination so that the driving screw rotates at 3·3 r.p.m. when the motor is operating at maximum speed. By means of a rheostat, rates of advance of 0·5 to 7 mm. per min. can be obtained. When the vaporisation furnace reaches the combustion furnace it is automatically stopped as the bearing runs on to a smooth section of driving screw.

Procedure—Place the vaporisation furnace 5 cm. from the combustion furnace, and place the sample so that half the boat is outside the movable furnace, on the side nearer the combustion furnace. Open the absorption tubes, turn on the heat to the vaporisation furnace, with the current adjusted to give a maximum temperature of 500° C. Allow the furnace to advance at 0·75 mm. per minute, for 4 min. then increase the rate to the maximum of 7 mm. per min. and maintain this rate until the sample has completely vaporised, after which decrease the rate to 0·75 mm. per min. After the sample has vaporised into the combustion tube again operate the motor at its maximum speed and raise the furnace temperature to 650° C. Three minutes after the bearing disengages, turn off the current.

Five slightly different cycles, covering a range of compounds boiling from 100° to 220° C., are illustrated. The accuracy of the semi-automatic unit is at least equal to that of the manual analysis, a conclusion borne out by many analyses performed over a number of years. The precision attainable is also satisfactory.

COMPARISON OF DEVIATION ON MANY ANALYSES

	No. of samples	Standard deviation parts per 1000	
		Carbon	Hydrogen
Semi-automatic ..	54	1·4	10
Power	218	2·5	18
Manual	76	1·4	9·1

Comparison of the combustion cycles employed by Hallett (*Ind. Eng. Chem.*, *Anal. Ed.*, **1938**, **10**, 101) and Royer *et al.* (*Ibid.*, 1940, **12**, 688) shows that (i) the Royer cycle, using a constant rate of advance throughout the combustion period, imposes a definite limitation as regards its application to compounds that must be vaporised under carefully controlled conditions, and (ii) Hallett's cycle should have a wider application, but it assumes that all compounds will have been vaporised into the combustion tube by the time the speed of the furnace is increased to a maximum; many cases are observed in which this is not so. Data indicate that with compounds that are volatilised with difficulty only the semi-automatic procedure gives accurate and consistent results. The semi-automatic unit was converted to fully automatic operation in which the five cycles could be selected by means of a five-gang, ten-circuit selector switch. The conclusions drawn from the semi-automatic unit are applicable to the fully automatic units.

H. K. DEAN

Analysis of Chloroacetic Acids. Determination of Dichloroacetic and Acetic Acid. G. A. Dalin and J. N. Haimsohn (*Anal. Chem.*, 1948, **20**, 470–472)—Most specifications issued by purchasers of chloroacetic acid include analytical methods for determining dichloroacetic acid based on the work of Pool (*Pharm. Weekblad.*, 1905, **42**, 165). The present authors have shown that the accuracy of this method is dependent on the quantity of reagent used; by standardising the quantities of reagents and by adding manganous sulphate the accuracy has been brought within the range required by the specifications' normal limit of approximately 2 per cent. of dichloroacetic acid. Determination of acetic acid by difference has also been shown to be unsatisfactory, and a direct method involving titration after distillation with an inert, intermediate-boiling solvent is proposed.

Determination of dichloroacetic acid—Dissolve 2·5 g. of the sample in 40 ml. of water, add 14 g. of sodium hydroxide pellets, and boil the solution under a reflux condenser for 1 hr. Cool, acidify with concentrated hydrochloric acid, neutralise with concentrated aqueous ammonia solution, and add 3 ml. of aqueous ammonia solution in excess. Concentrate the solution by boiling until crystals begin to form and, while hot, add 11 ml. of freshly prepared, 5 per cent. calcium acetate solution and 5 ml. of concentrated aqueous ammonia solution. Allow the mixture to stand, with occasional stirring, for 1 hr. and filter through a No. 40 Whatman filter paper. Wash the flask and the precipitate successively with 20 ml. of 10 per cent. aqueous ammonia solution, 20 ml. of 1 per cent. calcium acetate solution, and 20 ml. of water. Return the filter paper to the flask, add 200 ml. of 10 per cent. sulphuric acid, heat to 70° C., and add 3 ml. of 0·2 per cent. manganous sulphate solution. Titrate immediately with 0·1 *N* potassium permanganate from a micro-burette, and carry out a blank determination on the reagents. One ml. of 0·1 *N* potassium permanganate ≡ 0·12895 g. of dichloroacetic acid.

There must be no delay between the addition of the sulphuric acid and the titration: any delay increases the attack on the filter paper and results in an inaccurate blank.

Determination of acetic acid—Mix 50 ml. of the sample with about twice its volume of methyl *n*-amyl ketone and distil the mixture through a column about 60 cm. long and 10 mm. inside diameter, packed with 1/16-in. helices. Collect the distillate in small fractions, titrate each fraction with standard alkali, and calculate the acid as acetic acid. Continue until the ratio of acid determined to size of fraction is constant and plot the cumulative acid values against the cumulative distillate. Extrapolate the straight portion of the curve back to zero distillate to eliminate the effect of the acetic acid evolved in the distillation, and hence read the quantity of acetic acid present in the sample.

Other solvents proposed include xylene and di-*n*-butyl ether. The boiling point of all three solvents is intermediate between that of acetic acid (118° C.) and chloroacetic acid (189° C.) but, since methyl-*n*-amyl ketone is soluble in water, the use of this solvent avoids the necessity of extracting the acetic acid by shaking with water.

The method should be applicable to any series of homologous compounds for which a series of inert intermediate solvents can be found, and the accuracy can be improved by increasing the size of the sample and the amount of solvent.

A. H. A. ABBOTT

Absorptiometric Micro-determination of Chloralose in Alcoholic Solution. Preliminary Note. J. Delvenne and G. Barac (*Rec. Trav. Chim.*, 1947, **66**, 443–444)—An absorptiometric method, suitable for the determination of 50 to 200 µg. of chloralose, and based on a colour reaction with resorcinol in concentrated sulphuric acid, is proposed.

Procedure—To 2 ml. of a solution of chloralose in 94 per cent. alcohol add 4 ml. of resorcinol reagent (0·25 g. of resorcinol in 100 ml. of sulphuric acid of *d* 1·84) and mix well. Set aside for 5 min. and add 2 ml. of water. Allow to stand for 25 min. more and measure the absorption of the solution at 500 mµ. in a 0·5-cm. cell, using as a reference solution 2 ml. of 94 per cent. alcohol treated in the same manner as the test solution.

Read off the chloralose content of the test solution from a calibration graph prepared by using standard chloralose solutions.

H. J. CLULEY

Electrometric Ferrocyanide Method for Determining Sugars. E. T. Podlubnaya and P. S. Bukharov (*J. Anal. Chem. Russ.*, 1948, **3**, 131–136)—In the variations of the ferrocyanide method for determining sugars, difficulties arise in the observation of the end-point in dark-coloured solutions and in the different behaviour of certain indicators in hot and cold solutions. The electrometric method described avoids these difficulties.

METHOD—*Apparatus*—The electrodes are platinum wires fused into glass tubes. One of them, the reference electrode, is immersed in an alkaline 1 per cent. potassium ferrocyanide solution or 1 per cent. potassium ferricyanide solution that has been titrated with a sugar solution. It is contained in a tube with a tap at the bottom, and the whole passes through a hole in the rubber bung of a conical flask used as titrating vessel. Two further holes carry the indicator electrode and a connection to a burette, from which the sugar solution is delivered. The electrodes are connected through a tapping key to a galvanometer. A known volume of the standard potassium ferricyanide solution is placed in the conical flask.

Standardisation—Dissolve in water 4·75 g. of sucrose, dried over calcium chloride, heat the solution at 100° C. for 10 to 15 min. with 50 ml. of 2 per cent. hydrochloric acid solution to produce inversion, cool, neutralise with sodium carbonate, and dilute to 1 litre. For use, dilute the solution until it is exactly 0·2 per cent., and transfer a portion to the burette. In the conical flask place 40 ml. of 1 per cent. potassium ferricyanide solution and 10 ml. of 2·5 *N* sodium hydroxide. Insert the electrodes, heat the contents of the flask to the boiling point, and add the sugar solution dropwise with constant shaking, until the galvanometer needle swings to the other side of zero. When this reading has been obtained, further titrations may be carried out by adding most of the sugar solution required for a titration at the start. The titre of the solution is established on the figure obtained after adding at the start 85 to 95 per cent. of the required amount of sugar solution.

Procedure—Take an amount of the test solution corresponding to about 4·5 g. of sugar, add 50 ml. of 2 per cent. hydrochloric acid, boil, cool, neutralise, and titrate with it as described in the standardisation.

The method was compared with that of Bertrand on samples of pure sugars, raspberry and black-currant liquors, chartreuse, brandy, etc. Results agreed to about 1 part in 300. The optimum concentration of sugar in the solution used for titration is 0·2 per cent.

G. S. SMITH

Kjeldahl Determination of Nitrogen without Distillation. Application to Samples containing Phosphorus. K. Marcali and W. Rieman, 3rd (*Anal. Chem.*, 1948, **20**, 381–382)—The disadvantages of the formaldehyde method recommended by the authors (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 709; *Analyst*, 1947, **72**, 163) are the conversion of any phosphorus present from primary to secondary phosphate between the methyl red and phenolphthalein end-points, and the precipitation of the sulphates of barium and calcium and the hydroxides of iron and aluminium, which make the end-point less sharp. In the modification now described phosphorus is precipitated as zirconyl phosphate with the hydroxides of zirconium, calcium, barium, iron, and aluminium.

Method—Weigh a sample containing 10 mg.-equivalents of nitrogen and digest and dilute as in the general procedure (*loc. cit.*). Transfer quantitatively to a 250-ml. volumetric flask and add 15 ml. of sodium bromide solution, 5 ml. of a zirconyl chloride solution (for 150 mg. of phosphorus) prepared by dissolving 322 g. of the octohydrate in 600 ml. of *N* hydrochloric acid and diluting to 1 litre with *N* acid and 3 drops of methyl red indicator. Add 10 *N* sodium hydroxide until the colour is yellow and *N* sulphuric acid until it is just pink. Cool and dilute the solution. Filter through a rapid, fluted 15-cm. paper, discarding the first 5 ml., and pipette 100 ml. into a 250-ml. Erlenmeyer flask. Add 1 drop of methyl red solution and adjust with 0·1 *N* sodium hydroxide to the end-point. Read the burette and continue the titration as previously described.

Results—The method is accurate to within 1 in 175 on pure organic compounds containing 2 per cent. of phosphorus added as sodium phosphate, and to within 0·05 per cent. on blood samples containing between 3·5 and 7·5 per cent. of nitrogen as determined by the standard Kjeldahl method.

The modification has not been extended to the micro- and semimicro-scales. M. E. DALZIEL

Factors affecting the Determination of Furfural. G. A. Adams and A. E. Castagne (*Canad. J. Res.*, 1948, **26**, 314–324)—The known gravimetric and volumetric methods for determining furfural also measure the methyl-furfural and hydroxymethylfurfural derived from the cellulose, starch, hexose sugars, and methyl pentoses often associated with furfural-yielding substances in plant materials. The colorimetric method, based on the reaction between furfural and aniline in acetic acid solution, developed by Stillings and Browning (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 499–502; *Analyst*, 1940, **65**, 663–664), is specific for furfural, and the present authors have confirmed that methylfurfural and hydroxymethylfurfural do not interfere with the determination. They have also stabilised the colour by adding oxalic acid and sodium chloride. The modified procedure has been used to determine the furfural yield in such plant materials as wheat straw, wheat bran, corn cobs, oat hulls, soya bean hulls, peat moss, and sphagnum moss. The results were compared with those obtained by the use of the bromine oxidation method (Hughes and Acree, *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 123–124; *Analyst*, 1934, **59**, 430) and this procedure gave results consistently higher than those obtained by the colorimetric method. Since both methods yield identical results with pure furfural, it is contended that the titration procedure measures other substances, including methylfurfural and hydroxymethylfurfural, whilst the proposed colorimetric method gives an accurate determination of furfural from plant materials.

Procedure—Reduce the air-dried material to No. 40 powder and transfer a quantity of material expected to contain between 10 and 100 mg. of furfural to a two-necked, round-bottomed, 500-ml. flask. Add 100 ml. of 12 per cent. hydrochloric acid and 20 g. of sodium chloride. Fit a dropping funnel containing 12 per cent. hydrochloric acid to one neck of the flask, and connect the other neck, by all-glass joints, to a condenser leading to a flask fitted with a Kjeldahl-like trap partially filled with 12 per cent. hydrochloric acid. Boil the liquid in the flask, and heat so that the level in the flask is maintained at 100 to 125 ml. by the continual addition of 12 per cent. hydrochloric acid from the dropping funnel, and the distillate collects at the rate of 400 ml. per hour. To test for complete removal of furfural, after about 1·5 hr. with pure pentose and 2·5 hr. for plant materials, neutralise 1 ml. of distillate with sodium hydroxide solution and add 1 ml. of a reagent prepared by mixing 1 ml. of freshly distilled aniline, 10 ml. of water, and 9 ml. of glacial acetic acid. If no colour develops in 10 to 15 min., the test sample contains no furfural.

Dilute the distillate to a known volume, usually 200 ml., neutralise a 10-ml. aliquot with sodium hydroxide solution, and dilute to 100 ml. with water. Mix 5 ml. of freshly distilled aniline with 50 ml. of glacial acetic acid and 10 ml. of 20 per cent. sodium chloride solution. Add 5 ml. of 5 per cent. oxalic acid solution, 5 ml. of 10 per cent. disodium phosphate solution, and place the flask containing the aniline reagent in a water-bath at 20° ± 0·5° C. Cool the diluted furfural distillate to 20° C. and transfer 5 ml. to the whole of the aniline reagent and dilute the mixture to 100 ml. with water. Maintain the solution at 20° C. for 1 hr. in the dark and then read the transmittance in a suitable colorimeter. Simultaneously, prepare a blank of all reagents and read its transmittance as 100 per cent. Calculate the quantity of furfural in the sample by reference to a standard curve prepared by applying the above procedure to known amounts of pure furfural.

The accompanying table, besides illustrating the higher results obtained with the bromine-titration process, compares the potential value of some plant materials as sources of furfural.

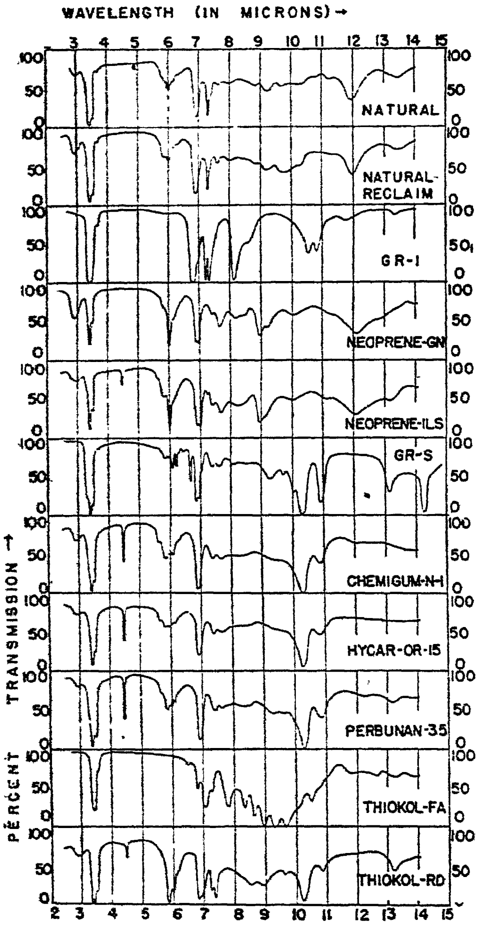
	Furfural, per cent.	
Material	Colorimetric method	Titration method
"Brewing" bran* ..	31·2	33·0
Corn cobs	24·9	27·1
Oat hulls	22·1	25·5
Wheat straw (crop I) ..	17·6	19·6
Wheat bran	17·2	18·9
Sunflower seed hulls ..	16·8	19·0
Wheat straw (crop II) ..	16·0	17·6
Flax shives	14·0	16·4
Milkweed pod hulls ..	9·8	13·3
Soya bean hulls	6·7	8·1
Peat (source, New Brunswick)	6·7	13·7
Sphagnum moss	5·3	9·4
Peat (source, British Columbia)	4·3	9·1

* A wheat bran product made by the patented Earle process and supplied for this work by the Continental Baking Co., Kansas City, Mo.

A. H. A. ABBOTT

Analysis of Natural and Synthetic Rubber by Infra-red Spectroscopy. H. L. Dinsmore and D. C. Smith (*Anal. Chem.*, 1948, **20**, 11–24)—The preparation of samples of raw and vulcanised rubbers for examination by infra-red spectroscopy is described. The identification of unknown rubbers is also described and directions are given for the quantitative determination of natural rubber and GR-S (the general purpose butadiene-styrene co-polymer) in admixture with each other, and the determination of the acrylo-nitrile content of oil-resisting rubbers. The relation between polymer structure and absorption spectrum is discussed.

Method—*Preparation of samples*—Extract unvulcanised rubber with a suitable solvent and then dissolve, either by 6 to 8 hr. shaking, or by mixing rubber and solvent in a mixing mill, in ethylene dichloride. Comminute vulcanised rubbers on a standard roll mill prior to extraction, and then dissolve by boiling under reflux in a suitable solvent having a boiling point above 100° C. Remove any fillers by centrifuging or filtration, concentrate the solution to a viscous mucilage, and spread between parallel metal spacing bars on a sodium chloride or potassium bromide plate. Allow the solvent to evaporate, leaving a dried film of the rubber. An appropriate film thickness must be found by trial, and should be such as to give 80 to 90 per cent. absorption at the 6·9-μ. band.



Spectra of Cured Elastomers

Spectroscopic measurements—The measurements reported were made on a large recording prism spectrometer of high resolving power. Two traces were recorded, one through the sample and the other through a blank plate. The ratios of corresponding ordinates were found. The percentage transmittances so obtained were reproducible to within ± 1·0 per cent. and the wavelength measurements to within ± 0·01 μ. from 2 to 15 μ.

Identification—Individual polymers can be identified from the reference spectra shown in the diagram.

Quantitative analysis—For mixtures of natural rubber and GR-S, measure the transmittance (T) as the ratio of the energy transmitted by sample and blank respectively, at 7·60, 7·25, and 6·70 μ. Duplicate measurements should be made, and transmittance values outside the range 20 to 65 per cent. should not be used. The extinction $E = \log (1/T) = KCt$, where K is a constant, C the concentration, and t the thickness. To eliminate the effect of the thickness, use extinction ratios and plot $E_{6\cdot70}/E_{7\cdot60}$ against GR-S content, and $E_{7\cdot25}/E_{7\cdot60}$ against natural rubber content for a series of standard samples, where the subscripts refer to the wavelengths used. Determine the composition of unknown samples from the calibration thus obtained. The presence of reclaimed natural rubber is found to interfere with the accuracy of the results, the error increasing with increasing amounts of reclaimed rubber.

The acrylonitrile content of the Buna N type rubbers is obtained by a similar method, the ratio $E_{4\cdot47}/E_{3\cdot50}$ being used. W. C. Wake

Inorganic

Potentiometric Determination of Small Amounts of Carbon Dioxide and Hydrochloric Acid in presence of each other in Distilled Water. Analytical Contribution for the Existence of Barium Bicarbonate. F. Cůta and R. Kohn (*Coll. Czech. Chem. Comm.*, 1947, **12**, 384–398)—Carbon dioxide can be determined in distilled water by potentiometric titration with barium hydroxide, using the antimony electrode. The method is suitable for concentrations of 0·01 to 5 mg.-mol. of carbon dioxide per litre.

In the presence of hydrochloric acid the sum of the hydrochloric acid and carbon dioxide is determined, necessitating the separate determination of the hydrochloric acid by another method. The simultaneous determination of hydrochloric acid and carbon dioxide is possible only within a narrow range of concentrations.

For the potentiometric titration of carbon dioxide alone the inflection point corresponds approximately to the formation of barium bicarbonate, and this fact is advanced as proof of the existence of barium bicarbonate in solution. However, if the inflection point is taken as the equivalence point, results for carbon dioxide are about 2 per cent. low; this effect is attributed to slight decomposition of the barium bicarbonate. It is necessary to determine the potential at the equivalence point by measuring the potential of solutions containing equivalent amounts of potassium bicarbonate and barium chloride, covering a range of concentrations. The equivalence potential thus determined varies with the concentration, and the potential to be taken for the end-point therefore depends upon the carbon dioxide content of the sample. By this method of titrating to a pre-determined potential carbon dioxide can be determined with an average error of 0·3 per cent.

Determination of carbon dioxide - barium hydroxide equivalence potentials—Introduce 500 ml. of water free from carbon dioxide into a flask in a current of pure air and add barium chloride and potassium bicarbonate in amounts equivalent to the formation of barium bicarbonate. Insert the antimony and saturated calomel electrodes and record the potential when it remains stable for 2 min. Repeat the procedure with barium bicarbonate solutions similarly produced covering a range of concentrations, and plot a curve of equivalence point potentials against corresponding carbon dioxide concentrations.

Determination of carbon dioxide in absence of hydrochloric acid—Weigh a flask containing sufficient paraffin oil to form a layer 1 to 2 cm. thick. Syphon in the sample below the oil and re-weigh to determine the quantity of sample taken. Introduce into the aqueous layer a stirrer, the tip of the burette, and the antimony and saturated calomel electrodes. Add 0·1 ml. of saturated potassium chloride solution per 100 ml. of sample to increase the conductivity.

Begin stirring, taking care that no oil is dragged down into the aqueous layer, and titrate with 0·01 *N* barium hydroxide solution. Calculate the approximate carbon dioxide content from the inflection point of the curve obtained and use this value to establish the equivalence potential from the equivalence potential curve. The amount of barium hydroxide added to give this potential is equivalent to the carbon dioxide in the sample.

The titration should be completed in 15 min., as in longer periods loss of carbon dioxide into the paraffin layer becomes significant.

Determination of carbon dioxide in presence of hydrochloric acid—Carry out the titration by the above method; two inflection points will be obtained. At the second inflection point the volume of barium hydroxide solution added will be equivalent to the sum of the hydrochloric acid and carbon dioxide present, subject to correction as above from the equivalence potential curve. To obtain the carbon dioxide content, determine hydrochloric acid alone, *e.g.*, by titration, in presence of methyl orange as indicator.

The first inflection point corresponds accurately to the hydrochloric acid content only under limiting conditions, when the hydrochloric acid and carbon dioxide contents are both in the region of 3 to 4 mg.-mol. per litre. H. J. Cluley

Determination of Magnesium in Dolomites by Photo-turbidimetric Titration. B. E. Reznik and G. P. Fedorova (*J. Anal. Chem. Russ.*, 1948, **3**, 92–95)—Most of the difficulties of nephelometry connected with the effects of various factors on the properties and physical structure of the precipitates obtained disappear when the method of photo-turbidimetric titration is used. This method has been applied to the determination of magnesium in the form of magnesium ammonium phosphate.

Development of the method—Preliminary experiments on the possibility of nephelometric determination of magnesium by means of di-sodium hydrogen phosphate in ammoniacal medium showed that although after 5 min. the optical density of the suspension became constant and the absorption strictly followed the Lambert-Beer law, yet the effect of temperature was too critical for the practical application of normal nephelometry. With photo-turbidimetric titration the equivalence point appeared at the same place at temperatures as far apart as 13° and 26° C., and it was unaffected by varying the waiting time after addition of each portion of the reagent solution over the range of 2 to 15 min. Calcium interfered but it could be removed as the molybdate. The molybdate is preferred to the oxalate method of precipitating calcium since the compound formed does not adsorb or occlude magnesium, thus rendering a double precipitation unnecessary, and the precipitate may be filtered off after a shorter time.

Procedure—Dissolve 0·5 g. of dolomite in 15 to 20 ml. of concentrated hydrochloric acid, previously treated with 0·5 to 1 ml. of concentrated nitric acid, evaporate to dryness, extract the residue with hydrochloric acid, boil, and filter. Add aqueous ammonia solution to precipitate the sesquioxides, dissolve and reprecipitate them, boil the combined filtrates, add 15 ml. of slightly ammoniacal 10 per cent. ammonium molybdate solution at the rate of 1 drop per sec., and then boil for 15 to 20 min. to coagulate the calcium molybdate. Cool, filter by suction, wash the precipitate six to eight times with hot water, and dilute the filtrate and washings to 250 ml. in a graduated flask. Transfer 5 to 10 ml. to a photo-turbidimetric cell, add 5 ml. of diluted aqueous ammonia solution (1 + 9) and half the calculated amount of disodium hydrogen phosphate solution. Then add during constant stirring 0·1-ml. portions of this solution at 2-min. intervals until the absorption is constant, as shown by the galvanometer needle. Determine the titre of the phosphate solution by similar means, using a solution of known magnesium content. One ml. should correspond to approximately 0·0016 g. of magnesium oxide.

With dolomites containing 10 to 20 per cent. of magnesium oxide, results agreed with those obtained by the pyrophosphate method to within 0·2 to 0·3 per cent. of the sample weight. G. S. Smith

Inorganic Drop Reaction for Copper. F. Goldschmidt and B. R. Dishon (*Anal. Chem.*, 1948, **20**, 373–374)—With concentrated hydrobromic acid cupric ions give a violet colour; the limit of concentration is 1 in 5 × 10^6.

Procedure—Place 1 drop of the test solution on copper-free filter paper (Green No. 803), and dry it in a warm air-current. Add 1 drop of a solution containing 5 g. of ammonium bromide and 4 ml. of syrupy phosphoric acid in 100 ml.; the appearance of a violet colour indicates the presence of copper. On adding a drop of water and drying in a warm air-current, a violet ring forms. In moist air the colour fades, but drying in warm air regenerates it.

In 0·05 ml., 0·2 μg. of copper can be detected in the presence of 12·5 parts of gold, or 25 parts of tervalent chromium, or 25 parts of ferric iron, or 125 parts of nickel, or 5 parts of cobalt, without special precautions; also 1 μg. of copper in 50 parts of bismuth.

Interfering substances—Mercurous mercury, stannous tin, and ferrous iron must be oxidised by boiling 1 drop of the test solution with 1 drop of saturated bromine water before applying the test.

Gold, bismuth, and ferric bromides colour the test solution when present in excess of the quantities stated above. If gold is present, boil 1 drop of the solution with 1 drop of the reagent and a few grains of silver and apply the test to the resulting solution; thus 0·2 μg. may be detected in presence of 500 μg. of gold. For bismuth, add 1 drop of 1 per cent. sodium acetate solution to 1 drop of the test solution and 1 drop of the reagent, and apply the test to the resulting suspension of bismuth phosphate: 0·2 μg. in presence of 500 μg. of bismuth can then be detected. For large amounts of iron, boil 1 drop of the solution with 1 drop of saturated bromine water, and add 1 drop of the reagent and 1 drop of 4 per cent. sodium fluoride solution. Apply the test to the cooled suspension of ferric phosphate; then 1 μg. can be detected in presence of 200 μg. of iron.

The photochemical colour change of silver compounds interferes, therefore boil 1 drop of the solution with 1 drop of the reagent to precipitate silver bromide, and to 1 drop of the supernatant liquid add a further drop of the reagent for the test; this method detects 0·2 μg. in presence of 2500 μg. of silver.

Chromium, cobalt, and nickel must be removed by ordinary analytical methods.

Oxidising acids give a yellow stain due to the liberation of bromine but, on drying, only the violet ring remains, except with iodates or periodates, which give a persistent stain. In this case, treat 1 drop of the solution with 3 drops of the reagent and dry in warm air; after some time a violet coloration develops, indicating copper. In this way 0·1 μg. of copper is detectable in presence of 1550 μg. of nitrate, and 0·5 μg. in presence of 2090 of chlorate, 3200 of bromate, 4380 of iodate, or 4800 of periodate. M. E. DALZIEL

Rapid Colorimetric Determination of Copper in Tin-base Alloys. G. Norwitz (*Anal. Chem.*, 1948, **20**, 469–470)—The sample is attacked by *aqua regia*, and the solution heated after addition of phosphoric acid to prevent precipitation of the tin on neutralising the solution. Water and ammonia are added and the resultant blue colour is measured in a Klett-Summerson photo-electric colorimeter with a 4-cm. glass absorption cell and a Klett-Summerson filter No. 59 (maximum transmittance at 580 mμ.)

Method—Treat 1·000 g. of the sample in a 500-ml. Erlenmeyer flask, marked to indicate the 500-ml. level, with 10 ml. of concentrated hydrochloric acid and then with 10 ml. of concentrated nitric acid; heat on a hot-plate to dissolve the sample. Add 30 ml. of 85 per cent. phosphoric acid solution and heat strongly until the solution is clear green in colour (12 to 15 min.). Allow to cool somewhat and add 200 ml. of cold water, followed cautiously by 120 ml. of concentrated aqueous ammonia while swirling. Cool the solution to room temperature, dilute it to the mark, stopper the flask, and shake it well. Read the blue cuprammonium colour in a colorimeter set to zero with distilled water. Deduce the copper content from a calibration curve prepared by using tin-base samples of known copper content.

Results—The method is suitable for copper contents between 1 and 10 per cent. and on 2 samples containing between 3 and 4 per cent. the values obtained were within ± 0·05 per cent. of the correct values.

The colour is stable for 24 hr. in a tightly-stoppered flask. Nickel, chromium, and cobalt interfere, but are usually absent in tin-base alloys; up to 5 per cent. of iron does not interfere. Tartaric acid does not keep tin dissolved in ammoniacal solution without the presence of phosphate. Tin and antimony are apparently not lost in driving off hydrochloric and nitric acids. M. E. DALZIEL

Concentration Methods in the Determination of Traces of Nickel. I. V. T. Chuyko (*J. Anal. Chem. Russ.*, 1947, **2**, 328–333)—Traces of nickel in aqueous solutions and in solutions of aluminium and zinc salts can be concentrated, prior to colorimetric determination, by co-precipitation with magnesium hydroxide. Traces in chromium and iron salts can be co-precipitated with zinc ferricyanide in acid solution, and traces in cobalt salts by addition of potassium cyanide and precipitation with sodium hydroxide and bromine water in presence of paper pulp.

Co-precipitation with magnesium hydroxide and colorimetric determination of nickel—To samples of nickel-free distilled water varying amounts of nickel equivalent to 0·001 to 0·1 mg. per litre were added, and the solutions treated with 40 ml. of 0·1 *M* magnesium sulphate and 20 ml. of 2 *N* sodium hydroxide per litre. After several hours, the clear supernatant liquid was siphoned off, the precipitate was dissolved in hydrochloric acid, its solution diluted to 0·1 *M* concentration in magnesium, and part of the magnesium precipitated with sodium hydroxide solution, and the solution filtered. For colorimetric determination of the nickel, the magnesium hydroxide was dissolved in a few millilitres of hot, 10 per cent. hydrochloric acid, the solution carefully neutralised with alkali, and then treated with 0·5 ml. of 0·1 *N* iodine in potassium iodide solution, followed by a saturated solution of sodium pyrophosphate with dimethylglyoxime, added until the precipitate first formed re-dissolved. In 5 to 10 min. the iodine colour disappeared and was replaced by the colour of the nickel complex. Sometimes it was necessary to add a small additional amount of solid sodium pyrophosphate to give a definite colour change. In further experiments the addition of magnesium salt and decantation by siphoning were replaced by filtration after addition of paper pulp.

Calcium, sodium, and potassium salts in concentrations of 1 g.mol. per litre had no effect on the co-precipitation, but ammonium salts required the addition of 2·5 g.-equivalents of sodium hydroxide for each gram-equivalent of ammonium salts for quantitative results. Colour matching was carried out against standards prepared in a similar way. The maximum error in the indicated range was 6 parts in 100.

Aluminium and zinc salts—Solutions containing known amounts of nickel were treated with 40 per cent. sodium hydroxide solution until the precipitated aluminium and zinc hydroxides were dissolved. After suitable dilution, paper pulp from a 7-cm. ashless filter paper was added, the solution well shaken and then filtered, and the paper with adsorbed nickel hydroxide washed two or three times with 2 *N* sodium hydroxide, and then with distilled water. Subsequent treatment was as before.

Apparently paper pulp was as good a collector of nickel hydroxide as magnesium hydroxide.

The error in determining 0·01 mg. of nickel in presence of 0·1 to 1 g. of aluminium or zinc did not exceed 5 parts per 100. In presence of chromium salts the magnesium precipitate did not settle properly and the cloudy solution passed through a filter. With paper pulp in place of magnesium hydroxide, no nickel appeared on the paper.

Co-precipitation with zinc ferricyanide—Solutions of chromium salts containing small amounts of nickel were acidified with a few drops of hydrochloric acid and heated to 40° C. For every 50 to 70 ml. of solution 2 ml. of 0·5 *M* potassium ferricyanide were added and then 10 ml. of 0·5 *M* zinc sulphate in drops. The precipitate was filtered off, washed with water to remove chromium salts, and then dissolved on the filter in 2 *N* sodium hydroxide. The filtrate was mixed with paper pulp and poured through the same filter which was then washed with alkali to remove ferricyanide. Further treatment was as described above. Quantitative precipitation of nickel occurred only when the zinc ferricyanide formed in presence of excess of ferricyanide.

Solutions of ferrous sulphate were freed from about 0·002 per cent. of nickel that was present, by oxidation with nitric acid, successive addition of 4 ml. of 0·5 *M* potassium ferricyanide and 4 ml. of 0·5 *M* zinc sulphate for each 70 to 100 ml. of solution, and filtration from the nickel-containing precipitate. To the weakly acid filtrates known amounts of nickel were added, and the solutions treated at 40° to 50° C. exactly as with chromium solutions. Some ferric hydroxide usually appeared during the alkali treatment. This could act as an efficient collector of the nickel unless the amount formed were very small, in which case the addition of paper pulp to the filtrate and re-filtration as described above were necessary. With 0·005 to 0·1 mg. of nickel in presence of 1 to 2 g. of iron, the results were normally slightly low but by not more than 6 to 8 parts in 100.

Determination in presence of cobalt—Solutions, 50 to 100 ml., of cobalt salts, containing small amounts of nickel, were treated with potassium cyanide solution until the precipitate first formed disappeared except for a slight cloudiness. They were cleared by heating and filtering. The filtrates were mixed with 10 ml. of 2 *N* sodium hydroxide, 75 ml. of bromine water, a further 25 ml. of alkali, and about 0·1 g. of paper pulp. After shaking for 15 to 20 min., they were filtered and the filters were washed with alkaline water. The precipitates were dissolved in hot, 20 per cent. hydrochloric acid solution and nickel was determined in the solutions. With 0·01 to 0·05 mg. of nickel in presence of 0·5 to 1·0 g. of cobalt the maximum error was 6 parts in 100. G. S. Smith

Colorimetric Determination of Small Quantities of Iron in Water by means of Dimethylglyoxime. P. Lieffrig and X. Buron (*Chimie Analyt.*, 1948, **30**, 36)—*Method*—To 100 ml. of the sample, in a Nessler tube with a ground-glass stopper, add 2 drops of concentrated hydrochloric acid and then a few crystals (10 to 20 mg.) of sodium dithionite ($Na_2S_2O_4$). Mix by inverting the tube a few times, add 8 to 10 drops of a 1·5 per cent. solution of dimethylglyoxime in alcohol, and mix again. Add 2 ml. of aqueous ammonia solution and mix once more. Compare the red colour produced (which is stable for several days) with standards of known iron content. The method works well if the iron content of the water is not above 1 mg. per litre. If the iron content is higher, a suitable volume of the sample should be diluted to 100 ml. with distilled water. A set of permanent standards can be made by dissolving amounts of cobalt chloride as given in the table in 100-ml. portions of 60 per cent. phosphoric acid.

mg. of $CoCl_2$ in 100 ml. of 60% phosphoric acid	Iron, mg. per litre
0·10	0·10
0·16	0·20
0·25	0·30
0·30	0·40
0·40	0·50

These standards should be checked with known iron solutions and adjusted if necessary: they may then be used for several years. L. A. Dauncey

Critical Examination of a Perchloric Acid Colorimetric Method of Analysis for Chromium in Carbon and Low Alloy Steel. Report of the Methods of Analysis Committee of the Metallurgy Division of The British Iron and Steel Research Association (*Metallurgia*, 1948, **37**, 211–216)—The method makes use of the optical absorption of dichromate, which is increased in presence of iron perchlorate. The effect of various factors on the results is given, and it is concluded that the method is not suitable as a standard one, but should be valuable for routine analysis, provided that the limitations are appreciated. The following findings have been taken into account in deciding the experimental conditions, which should be closely controlled.

(1) The absorption is substantially independent of perchloric acid concentration within the range 34 to 44 per cent.

(2) The colour system behaves as if it had two components, one due to the chromium alone and

the second being an intensification proportional to the amount of iron in the solution.

(3) Of the common alloying elements, only molybdenum and vanadium in amounts greater than 1 per cent. interfere. The use of sulphuric or phosphoric acid should be avoided.

(4) The optical absorption is influenced by the temperature of the solution, which should be 20° ± 2° C. during the measurement.

(5) The use of more ferrous sulphate than the suggested amount causes low results.

(6) Filtration of the solution is not necessary when the silicon content is low (0·1 to 0·2 per cent.). When filtration is necessary a sintered-glass and not a paper filter should be used.

Procedure—Dissolve 1 g. of sample in 10 ml. of diluted nitric acid (1 + 1) and 23 ml. of perchloric acid (sp.gr. 1·54) in a covered, 200-ml. conical beaker). (A 0·5 g. sample may be used, and the volume of perchloric acid is then reduced to 18 ml.). Evaporate the solution to perchloric acid fuming and boil gently for 5 min. Cool rapidly, add 20 ml. of distilled water to dissolve the salts, and cool again. Dilute exactly to 50 ml. and measure the absorption in a Spekker absorptiometer, using a water : water setting of 1·20. With 1·0-g. samples and chromium contents less than 0·15 per cent. use 2-cm. cells, and for chromium contents between 0·15 and 0·35 per cent., use 1-cm. cells; with 0·5 g. samples and chromium contents less than 0·1 per cent., use 4-cm. cells and for chromium contents between 0·1 and 0·4 per cent., use 2-cm. cells. With a mercury vapour lamp use either Chance No. 6 and Wratten No. 50 filters or Ilford 601 and H.503 filters. With a tungsten lamp use Ilford 601 and H.503 filters. Reduce the solution (either that in the cell or a further portion) with 0·02 to 0·04 g. of ferrous sulphate and measure the absorption immediately. The slope of the line connecting the Spekker difference readings and the chromium content depends on the iron content of the solution.

L. A. DAUNCEY

New Method for Determining Tungsten in Steels by means of β-Naphthaquinoline. R. B. Golubtsova (*J. Anal. Chem. Russ.*, 1948, **3**, 118–122)—β-Naphthoquinoline can be used instead of cinchonine for the determination of tungsten in steels, ferro-tungsten, and pure tungsten. Results of the same order of accuracy are obtainable.

Properties of β-naphthaquinoline—Preliminary experiments were carried out by adding 5 to 10 ml. of a 2 per cent. aqueous solution of the reagent acidified with sulphuric acid to solutions containing various metallic ions in acidic and alkaline media. No reaction occurred with ferrous iron, nickel, aluminium, titanium, zinc, lead, niobium, nitrate, or chromate. Molybdenum gave a dense white precipitate in neutral or weakly acidic solution, but none in more acidic solutions. The reagent is practically insoluble in water. On being heated with very dilute hydrochloric acid, it dissolves and is re-precipitated in the cold as yellow needles. These after filtration and drying can be dissolved in water to give a 2 per cent. solution. Both alcoholic and acetic acid solutions of the reagent give only colloidal suspensions with soluble tungstates. The reagent dissolves readily in water acidified with sulphuric acid; a 2 per cent. solution can be kept unchanged for a long time in an unsealed vessel. This solution gives precipitates with tungstates in both hot and cold solutions and, although heating may be used to coagulate the precipitate, this is not essential. The precipitate that has been formed in the cold may be filtered through a close filter after 10 to 15 min. standing. Results expressed in the form of a Gibbs triangular diagram showed that 1 ml. of a 2 per cent. solution of the reagent precipitates 29·6 mg. of tungsten in the presence of 1 ml. of 10 per cent. sulphuric acid solution.

Determination of tungsten in steels—Dissolve 1 to 2 g. of steel in a covered beaker by heating with 80 ml. of diluted hydrochloric acid (1 + 4), followed by the addition of a few drops of nitric acid. Heat until the tungstic acid that separates is bright yellow, then add 60 ml. of water, and heat again for 20 to 30 min. Cool, add 10 ml. of 2 per cent. β-naphthoquinoline in water containing sulphuric acid, filter after 15 min., wash first with 10 per cent. hydrochloric acid solution until a test shows that all iron has been removed, and then with 2 per cent. ammonium nitrate solution. Ignite the precipitate at first at a low temperature and then at 800° C. to tungstic oxide (factor 0·7931).

If the precipitate is contaminated, fuse it with 3 g. of potassium-sodium carbonate mixture, extract with water, acidify the solution with diluted hydrochloric acid solution (1 + 1), and re-precipitate etc. as before. [Treatment with hydrofluoric acid may be advisable. G.S.S.]

Determination in ferro-tungsten and metallic tungsten—Treat 0·5 g. in a platinum dish with 10 ml. of hydrofluoric acid and 10 ml. of concentrated nitric acid added in drops. After dissolution, add 2 to 3 ml. of concentrated sulphuric acid and heat for 20 min., avoiding the deposition of salts. Transfer the clear solution to a beaker, add 10 ml. of the reagent solution, and evaporate on asbestos to a volume of 50 to 60 ml. Cool, filter, and proceed as described above for steel.

[ABSTRACTOR'S NOTE—Tables in the paper intended to show accuracy give, for steels, two columns, "W% taken" and "W% found," *e.g.*, taken 1·24%, found 1·25, 1·20, 1·21. The "taken" figure might be that obtained by the cinchonine method, but there is nothing to show this. The tables are not referred to in the text, but there is a statement that the accuracy is as good as that of the cinchonine method.] G. S. SMITH

Electrometric Determination of Vanadium in Steels. A. Claassen and J. Corbey (*Rec. Trav. Chim.*, 1948, **67**, 5–10)—In the volumetric method of Thanheiser and Dickens (*Arch. Eisenhüttew.*, 1931, **5**, 105) steel is dissolved in a mixture of sulphuric and phosphoric acids, and carbides are oxidised by adding potassium permanganate. Chromium and vanadium are reduced by means of ferrous sulphate and sufficient permanganate is added to the cold solution to give a lasting faint colour. The small excess of permanganate is destroyed by adding oxalic acid and the vanadium is titrated electrometrically with ferrous sulphate solution. This method gives low results, probably because the oxalic acid reduces some vanadium, and other reducing agents have been used without success. The method of the A.S.T.M. (1946) in which the steel solution is reduced with ferrous sulphate, excess of which is destroyed by means of ammonium persulphate, and the vanadium is titrated with potassium permanganate solution, also gives low results.

The method now described is claimed to be satisfactory. Excess of oxidising or reducing agent is removed by means of the titrant and the amount of vanadium is determined by the amount of reagent consumed between two electrometric end-points. By the determination of formal redox potentials and other experimental work, conditions have been found that make both end-points sharp.

Methods—Vanadium in tungsten-free steel—Dissolve up to 1 g. of steel in 6 ml. of phosphoric acid (sp.gr. 1·7), 8 ml. of diluted sulphuric acid (1 + 1), and 20 ml. of water. When the vanadium content is small, use up to 4 g. of steel and 3 ml. more of diluted sulphuric acid (1 + 1) for each extra gram of steel. Dilute to 70 ml., boil, add 2·5 per cent. potassium permanganate solution until the solution is strongly coloured and boil for a few min. Add solid ferrous sulphate in small portions until no further colour change occurs. Dilute to 200 ml., cool to below 30° C. and add, with constant stirring, potassium permanganate solution until a small excess is present. Add 2 to 4 drops in excess and leave for 2 min. to make certain that the colour persists. Titrate electrometrically with 0·02 to 0·1 *N* ferrous sulphate solution. When the first equivalence point is reached (0·90 to 0·95 v. with respect to the saturated calomel electrode) add 40 ml. of diluted sulphuric acid (1 + 1) and continue the titration to the second end-point (0·62 to 0·67 v. *versus* S.C.E.).

Vanadium in tungsten steels—With moderate amounts of vanadium in the presence of tungsten the above method can be used if the amount of phosphoric acid is increased to 30 ml., but the first end-point is not sharp. The following method is preferred. Dissolve the steel in 16 ml. of diluted sulphuric acid (1 + 1), 20 to 40 ml. of water, and 6 ml. of phosphoric acid for up to 1 g. of sample, 14 ml. for 2 g. and 22 ml. for 3 g. These quantities are adequate for steels with up to 25 per cent. of tungsten. Heat until the evolution of hydrogen ceases and then add 2·5 per cent. potassium permanganate solution in excess and boil until the evolution of gas ceases. Dilute to 75 ml., and add solid ferrous sulphate to the hot solution until the colour of the solution shows no further change. Boil for 5 min. to effect quantitative reduction of the vanadium from the vanadophosphotungstate complex, cool, dilute to 175 to 200 ml., and titrate electrometrically with 0·02 to 0·1 *N* potassium permanganate to the first end-point (0·58 to 0·63 v. *versus* S.C.E.). Dilute the solution to 350 to 400 ml. (600 ml. for a 3-g. sample), heat to 60 to 70° C., and continue the titration to a second end-point at 0·84 to 0·92 v. *versus* S.C.E.

L. A. Dauncey

Composition of the Phospho-vanado-molybdate Complex Obtained in Colorimetric Determination of Phosphorus by Misson's Method. N. V. Maksimova and M. T. Kozlovsky (*J. Anal. Chem. Russ.*, 1947, 2, 353—358) —Misson (*Chem.-Ztg.*, 1908, 32, 633) used the yellow coloration given by phosphates in acid solution with ammonium metavanadate and molybdate for the colorimetric determination of phosphorus. He ascribed to the complex formed the formula $(NH_4)_3PO_4,NH_4VO_3,16MoO_3$ largely on the basis of determinations of phosphorus and vanadium in precipitates from more concentrated solutions. This formula, which has been taken as correct by other workers, is not, however, in accordance with the theory of the structure of complex compounds of phosphorus and molybdenum.

It is now shown that the formula is wrong. The complex contains no ammonium, and the ratio $P_2O_5 : V_2O_5 : MoO_3$ is 1 : 1 : 22. Attempts at separating the complex from the medium in which colorimetric determinations of phosphorus are carried out involved a search for precipitants and a method of extraction with a solvent. Barium chloride, silver nitrate, mercurous nitrate, and magnesium chloride gave no precipitate, but 8-hydroxyquinoline gave an orange precipitate of the composition $6C_9H_7ON.P_2O_5.V_2O_5.22MoO_3.nH_2O$, in which *n* varied between 12 and 35. Analyses for molybdenum, vanadium, phosphorus, and 8-hydroxyquinoline were made, and it was established incidentally that weighing of the precipitate might be used for gravimetric determination of phosphorus, and titration of the 8-hydroxyquinoline present for volumetric determination of phosphorus. Extractions were attempted with *iso*amyl alcohol, ether, and chloroform. Chloroform had no effect on the complex or its components, but the other solvents removed the complex entirely in one extraction. They also extracted much of the excess of molybdate present, but only traces of vanadate and these could be removed from the extract by two or three washings with *N* nitric acid saturated with *iso*amyl alcohol. Phosphate was not extracted. Thus, the extraction method could be used to give the ratio $P_2O_5 : V_2O_5$, but no further information. Analysis of the residue after evaporation of the *iso*amyl alcohol extract gave this ratio as 1 : 1.

An indication that no components other than P_2O_5, V_2O_5, MoO_3, H_2O, and possibly hydrogen were present, was obtained by using only phosphoric acid, vanadium pentoxide, soluble molybdic acid, and nitric acid. The coloration was exactly the same as that obtained under normal conditions. The complex is represented as $[P_2(V_2O_6)(Mo_2O_7)_{11}]$.

Formation of the 8-hydroxyquinoline compound and its analysis—To separate 1-, 2-, and 3-mg. quantities of phosphorus in the form of potassium dihydrogen phosphate, there were added 10 ml. of 0·02 *M* ammonium metavanadate in 0·125 *N* nitric acid, 5 ml. of 10 per cent. ammonium molybdate solution, 10 ml. of diluted nitric acid (sp.gr. 1·2), and water to make 50 ml. Then 20 ml. of 1 per cent. 8-hydroxyquinoline solution in alcohol were added in the cold with stirring. Precipitation was immediate. After a test for complete precipitation the solution was filtered through a porcelain filtering crucible, and the precipitate washed with 0·2 per cent. 8-hydroxyquinoline solution in *N* nitric acid. Washing with water would have caused the precipitate to run through the filter. The crucible was dried to constant weight at 100° C. An aliquot part of the dried precipitate was further ignited to constant weight at 450° C.

The ignited precipitate was dissolved by heating in concentrated hydrochloric acid, followed by addition of water to give an acid concentration of 15 per cent. Then 3 g. of tartaric acid were added to combine with the vanadium, the solution was boiled, a rapid stream of hydrogen sulphide was passed through it for 15 min., an equal volume of water was added, the passage of gas continued for 10 min. more, the solution was boiled for 3 min., and hydrogen sulphide passed for another 10 min., whereupon the solution was left to stand in the cold for 1 hr. The precipitated molybdenum sulphide was filtered off and washed with a 2 per cent. solution of tartaric acid in 1 per cent. hydrochloric acid saturated with hydrogen sulphide. It was then dissolved in ammonia solution and transformed to lead molybdate. The filtrate was boiled, and oxidised with ammonium persulphate, and the remaining traces of molybdenum were precipitated by hydrogen sulphide. After filtration the solution was evaporated to dryness with nitric acid, and the residue ignited and dissolved in sodium hydroxide solution. The solution was neutralised with nitric acid and 5 per cent. in excess was added, and the vanadium was determined colorimetrically with hydrogen peroxide. The solution was then boiled to remove hydrogen peroxide, vanadium was reduced with sulphur dioxide solution, and phosphorus determined as magnesium pyrophosphate.

For the determination of 8-hydroxyquinoline the complex dried at 100° C. was dissolved by heating in a mixture of alcohol and hydrochloric acid, the solution was mixed with an equal volume of water, cooled, treated with an excess of standard potassium bromate-bromide solution, and then with potassium iodide, and the liberated iodine titrated with thiosulphate solution in presence of starch. [No further details are given.]

Results showed that the weight of the ignited residues and the amounts of 8-hydroxyquinoline found were proportional to the weights of phosphorus taken. Hence the methods described above up to the weighing of the ignited complex and the titration of 8-hydroxyquinoline in the dried complex may be used for gravimetric and volumetric determinations of phosphorus. The gravimetric factor is 0·0174 and the volumetric factor is 0·000259 for 1 ml. of 0·1 *N* potassium bromate. The actual results were: 1·0 mg. of phosphorus gave 60·0 and 58·4 mg. of ignited precipitate and 14·08 and 14·04 mg. of 8-hydroxyquinoline, 2·0 mg. gave 115·0 and 114·8 mg. and 28·02 and 28·04 mg and 3·0 mg. gave 174·0 and 168·0 mg. and 42·05 and 42·09 mg., respectively. G. S. Smith

Determination of Molybdenum in Ferro-Molybdenum by a Potentiometric Method. H. Wirtz (*Z. anal. Chem.*, 1939, **116**, 240–243)—Previous methods use the precipitation of molybdenum as its sulphide or as lead molybdate after separation of the iron by sodium peroxide fusion. Molybdenum is so easily reduced by impurities that quantitative precipitation of the sulphide is difficult and the precipitate obtained is not easy to handle; in the fusion some molybdenum is lost by co-precipitation with iron and chromium hydroxides. The potentiometric method described entails reduction of hexa- to penta-valent molybdenum by tervalent titanium. The apparatus consists of a saturated calomel reference electrode, a platinum gauze indicator electrode, connected through a galvanometer, and a motor-driven stirrer led through the gauze. The potential of the standard electrode is such that the end-point is indicated by zero deflection of the galvanometer needle. The titanous chloride solution must be added from a burette with 0·05-ml. graduations. Chromium, at the concentration present in ferro-molybdenum, does not interfere.

Titanous chloride solution—Treat 80 ml. of a 10 to 15 per cent. solution of iron-free titanous chloride with 300 ml. of concentrated hydrochloric acid and then dilute to 5 litres.

Standardise the solution by dissolving copper oxide equivalent to 1 g. of copper, checked electrolytically, in 30 ml. of hydrochloric acid and diluting the solution to 1 litre. Dilute 50 ml. of the solution to 200 ml. in a 600-ml. beaker, and add 10 ml. of 15 per cent. potassium thiocyanate solution, and 10 ml. of diluted hydrochloric acid (1 + 1). Titrate with titanous chloride as described below. The concentration towards molybdenum is obtained by multiplying the concentration towards copper by 1·51.

Procedure—Fuse 1 g. of the sample with 20 g. of sodium peroxide in an iron crucible and extract the cooled melt with water in a beaker. Boil the solution with sodium peroxide for 3 to 5 min., then cool, and dilute to 500 ml. in a graduated flask. Evaporate the solution to perchloric acid fuming Pipette 50 ml. of the clear liquid into a 600-ml. beaker and dilute to 200 ml. with water. Add 10 ml. of 15 per cent. potassium thiocyanate solution, neutralise to litmus with diluted hydrochloric acid (1 + 1), and add 10 ml. in excess. Place the beaker in the apparatus and start the titration. Add 3 to 5 ml. of the standard titanous chloride solution dropwise and then continue the addition in a stream until disturbance of the galvanometer needle shows equivalence is approached. Continue adding the reagent dropwise until the galvanometer reads zero.

To clean the apparatus stir with aqueous ammonia solution for 1 min., and then rinse the electrodes thoroughly with water to remove the titanium hydroxide.

Results—Compared with 7 results obtained by the sulphide method, agreement is to within 1 in 380; and with 9 results by the molybdate method, to within 1 in 680. M. E. Dalziel

Quantitative Determination of Lead in Presence of Cations of the Second Analytical Group [Barium, Strontium, Calcium, and Magnesium]. V. P. Shvedov, E. O. Goldshteyn, and N. I. Seletkova (*J. Anal. Chem. Russ.*, 1948, **3**, 109–112)—Existing methods of separating lead from the alkaline earths and magnesium are unsatisfactory. It is now shown that quantitative separation occurs when lead is precipitated as the hydroxybromide or hydroxyiodide. Determination of lead can then be made by the chromate method. Completeness of precipitation of lead was proved radiometrically and also by tests with hydrogen sulphide. In the former case the radioactive isotope ThB was added to the original lead solution as an indicator. Precipitation must be carried out at room temperature, and the precipitate filtered after not less than 1 hr. The ratio of bromide or iodide ions to hydroxyl ions in the solution should be 2 : 1.

Procedure—(Tests were carried out on solutions containing lead acetate and the nitrates of the other metals). To a solution containing barium, strontium, calcium, magnesium, and lead, add a mixture of equal volumes of 2 *N* ammonium bromide or iodide and *N* aqueous ammonia solution at room temperature. After 1 hr. filter off the precipitate and wash it five times with the precipitating solution diluted ten times. Treat the precipitate on the filter three times with boiling ammonium acetate solution, and then wash with hot water, collecting the filtrate in the original beaker. Dilute the filtrate with water, boil for 5 min. with 10 ml. of concentrated potassium dichromate solution, cool, filter through a filtering crucible, wash with water acidified with acetic acid, dry the lead chromate at 100° to 110° C., and weigh.

Tests carried out with 0·1 g. of lead in the presence of equal and five- and ten-fold quantities of the individual alkaline earths and in the presence of equal quantities of all the elements together gave results correct to 0·5 parts or less in 100.

G. S. Smith

Polarographic Analysis of Refined Lead. M. Zotta (*Gazz. Chim. Ital.*, 1948, **78**, III, 143)—The principal metals that it is necessary to determine in lead used for accumulators are antimony, bismuth, arsenic, iron, copper, silver, tin, and zinc. For silver, arsenic, and tin, the spectrographic method is the most suitable. The remaining metals and cadmium and nickel can be rapidly and accurately determined polarographically.

Procedure—Dissolve 10 g. of lead shavings freed from grease, in a graduated 100-ml. flask, in 50 ml. of pure nitric acid (sp.gr. 1·3) diluted with three parts of water. If traces of tin or more than 0·03 per cent. of antimony are present, the solution will not be perfectly clear.

Add to the liquid 5 ml. of concentrated sulphuric acid, dilute to the mark with water, mix, and allow to stand for about 30 min.

(*a*) To determine copper, zinc, cadmium, and nickel, pipette 20 ml. of the clear liquid from above the lead sulphate precipitate into a small dish of Jena glass and evaporate to dryness, heating cautiously with the naked flame. Dissolve the residue in 3 ml. of a solution having the composition –$(NH_4)_2SO_4$, 1·5 *N*, $(NH_4)_2CO_3$, 0·1 *N*, NH_4OH, 0·5 *N*, and gelatin 0·005 per cent. After eliminating the dissolved oxygen from the solution, obtain the polarogram. The first step obtained is due to copper, the second to the small amount of lead remaining in solution, and the following steps to cadmium, nickel, and zinc, in this order.

(*b*) To determine iron and bismuth, take 40 ml. of the clear liquid, evaporate first on the water bath and then by cautious heating with the naked flame until sulphuric fumes are evolved. Pour the liquid into a centrifuge tube and wash the dish with 2 ml. of water. Precipitate the hydrates by addition of concentrated aqueous ammonia solution drop by drop, heat for 20 min. on the water-bath, cool, and centrifuge. Wash the residue twice with a small amount of dilute aqueous ammonia solution. Dissolve in 1·5 ml. of 4 *N* sulphuric acid. Add 3 ml. of *M* sodium citrate, a drop of methyl red, and aqueous ammonia solution drop by drop until the colour change begins. Obtain the polarogram. The step due to iron is followed by that of bismuth and then by that due to the sum of lead and antimony present.

(*c*) To determine antimony, take 5 ml. of the clear liquid and obtain the polarogram directly. If the amount of antimony exceeds 0·02 per cent. the results will be low, since part of the antimony remains undissolved by the nitric acid.

Prepare standards by adding the various metals to lead whose impurities are known, uniformity of the alloy being secured by prolonged heating at 550° C. in vacuum in a sealed glass tube with continuous oscillation.

The maximum sensitivity is 0·0005 per cent. for cadmium, bismuth, and iron; 0·0004 per cent. for copper; 0·0002 per cent. for zinc; and 0·001 per cent. for antimony. The average errors are ±8 per cent. for antimony, ± 6 per cent. for copper, and ± 3 per cent. for all the others.

A. H. Bennett

Precious Metal Sulphide Series. G. A. Medvedeva (*J. Anal. Chem. Russ.*, 1948, **3**, 103–108)—The order of decreasing solubility of the precious-metal sulphides in solutions of salts of these metals is Ir_2S_3, Rh_2S_3, PtS_2, Ru_2S_3, OsS_4, PdS, and Au_2S_3. Osmium, palladium, and gold can be completely precipitated as sulphides by 10 min. boiling of solutions of these metals with sulphides of iridium, rhodium, platinum, or ruthenium. A platinum solution can thus be freed from impurities of osmium, palladium, and gold; it is only necessary to treat the solution with platinum sulphide.

G. S. Smith

Physical Methods, Apparatus, etc.

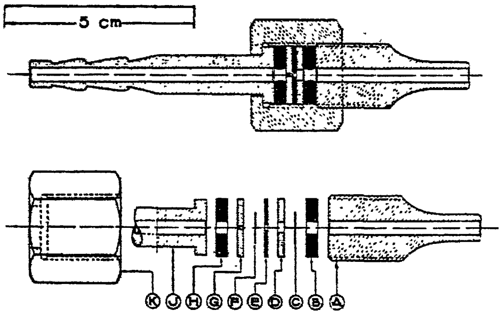
Measurement of Water in Gases by Electrical Conduction in a Film of Hygroscopic Material and the Use of Pressure Changes in Calibration. E. R. Weaver and R. Riley *J. Res. Nat. Bur. Stand.*, 1948, **40**, 169–214)—The electrical conductivity of a thin film of phosphoric acid alters considerably with alteration in concentration of water vapour. For example, the resistance of such a film changes from 2870 × 10^6 ohms for a partial pressure of water vapour of 0·011 mm. of mercury to 8×10^8 ohms for a partial pressure of water vapour of 20·2 mm. If two samples of gas, one containing a known concentration of water vapour, S, and one unknown, W, are adjusted to give the same electrical resistance across a hygroscopic film by varying the pressure of the gases to P_S for the standard gas and to P_X for the unknown gas, then the concentration of water vapour in the unknown gas can be obtained from the simple relationship $W = S/P_S P_X$, assuming no deviations from Boyle's and Dalton's laws. To allow for such deviations, another and more complicated equation has been deduced, but circular slide rules have been developed to simplify calculations.

The apparatus is described in detail, especially the film detectors of various forms, electrical indicators, saturators, and valves. A discussion is given of the effectiveness of different electrolytic films, made from phosphoric and sulphuric acids and from lithium chloride in glycerol and other solvents.

The apparatus appears to have been extensively used for determining moisture in oxygen used by aviators, in dichlorodifluoromethane, in air, in powdered solids, and in organic liquids, and for testing the capacity of drying agents. The pressures used have mostly been in excess of atmospheric pressure but the authors think that the method might be used at low pressures also. They further suggest the method should be particularly useful for detecting oxygen in a combustible gas or *vice versa* by means of the water produced on combustion.

W. J. Gooderham

Membrane Valve for Constant Gas Flow. G. W. Harmsen (*Chem. Weekblad*, 1947, **43**, 205–206)—Needle valves and similar means for regulating the flow of gas do not allow of a small rate of flow being kept constant over a long period of time without adjustment. The author therefore used a "membrane valve," the construction of which is evident from the figure. B and H are thick rubber rings with large openings, whilst E is a thinner ring with a smaller opening, larger, however, than that in the metal plate G. This ring, E, presses the membrane F against the plate G, which is bored with a hole of about 0·3 mm. diameter. E should not be too thick or soft. Before the gas passes through the flow-controlling membrane, F, it passes through a similar membrane, C, on the plate D, which has a much larger opening. This serves as a filter. The membranes are cut out of paper—thick filter paper for rapid gas flow and sized writing paper for lower rates.



G. Middleton

The Critical Air-blast Test. F. G. Kreulen, van Selms, and D. J. W. Kreulen (*Chem. Weekblad*, 1948, **44**, 208–211)—The "critical air-blast test" (Methods of Test of Transport Gas Producer Fuel, B.S. 1264, 1945) determines the suitability of a coke for various purposes, including its use as household fuel. The test is defined as the minimum blast rate, measured in cubic feet per minute, at 60° F. and 30 in. mercury pressure (dry gas), that will maintain combustion in a fully ignited bed of dry fuel 40 mm. in diameter, and not less than 25 mm. deep, for a period of not less than 20 min. Combustion is judged to be maintained if, after the blast rate is increased to 0·150 cu.ft. per min., a visible glow appears within 20 min.

For household coal, the value should be not more than 0·055. Samples of coke made from coals of different origins all showed values between 0·06 and 0·08, but there was no clear relation between the degree of coking of a sample of coal and the critical air blast. A number of abnormal cokes gave the following values:—

Sample	Ash per cent.	Volatile matter per cent.	Critical air blast
Petroleum coke ..	0·1	8·1	0·034
Retort graphite ..	0·6	0·0	0·118
Peat coke (war-time production) ..	6·2	35·5	0·004
Coke from high-volatile non-coking coal	4·3	5·1	0·031
Coke from wood ..	1·0	1·9	0·026
Coke from peat ..	3·5	3·8	0·024
Coke from Dutch brown coal	13·1	6·7	0·020
Coke from anthracite	5·8	2·8	0·086
Coke from Cannel coal	11·6	4·2	0·033

With peat coke, the first sample was made at low temperature, but present methods of production use a high temperature of 1050° to 1100° C. With anthracite and similar coals, the critical air blast shows a direct relation to the amount of volatile matter; anthracites with a low content of volatile matter give a high critical air blast value; this figure thus should be useful for evaluating such samples. There appears to be a straight-line relationship between the critical air blast and the temperature at which the coke is made. This dependence on temperature is greater with anthracites than with high-volatile, non-coking, coals. When cokes with different values for the critical air blast are mixed, the curve obtained is not quite rectilinear but somewhat S-shaped. This indicates that household cokes are improved by the addition of anthracite; it appears probable that petroleum coke would also be a useful addition.

G. Middleton

Polarographic Behaviour of Ions with Sodium Fluoride as Supporting Electrolyte. P. W. West, J. Dean, and E. J. Breda (*Coll.*

Czech. Chem. Comm., 1948, **13**, 1–10)—The polarographic behaviour of a number of inorganic ions, using sodium fluoride as supporting electrolyte, has been investigated to find whether the complex-forming properties of sodium fluoride have an important effect on the reduction waves of these ions. Of the twenty ions reported upon, only three, cobalt, tin and antimony, give steps whose half-wave potentials are significantly different from the corresponding potentials obtained when hydrochloric acid or potassium chloride is used as supporting electrolyte. J. G. Waller

Determinations with Radioactive Isotopes by Measuring Specific Activity. P. Süe (*Bull. Soc. Chim.*, 1947, 405–406M)—In a solution containing a mixture of ions, an ion can be determined by adding a known weight of its radioactive isotope, converting the active and inactive ions into an insoluble salt and measuring the specific activity (number of particles emitted per minute per unit weight) of the precipitate. If the specific activity of the added isotope in the form of the insoluble salt is known, the observed reduction in specific activity due to its dilution with the inactive salt can be used to calculate the concentration of the ion originally present. The advantage of the method is that the precipitation need not be quantitative, as the proportion of active to inactive ion will remain the same, and the method should be of special value in the determination of traces where solubility errors might otherwise be significant. The precipitate must be free from impurity.

Results are given for the determination of potassium in presence of 13 g. of sodium and lithium nitrates. Single precipitations yielded potassium perchlorate of adequate purity, and results for quantities of 0·1 to 1 g. of potassium normally showed less than 2 per cent. error. Accurate results were not obtained under these conditions for smaller quantities of potassium. H. J. Cluley

Reviews

Commercial Fruit and Vegetable Products. By W. V. Cruess. Third Edition. Pp. x + 906. London: McGraw-Hill Publishing Co., Ltd. 1948. Price 51s.

This third edition of Professor Cruess's textbook, *Commercial Fruit and Vegetable Products*, brings up to date the industrial and technological information on the subject, and includes a new chapter (XXXV) on Plant Sanitation, which though useful, and valuable in drawing attention to this very important essential in the preparation of any food material, might well have been given more space than the 19 pages allotted to it.

Anyone closely familiar with a particular industry can find some points of criticism in a textbook on that subject, but any such criticism of this work would be more carping than justified. If there is a genuine criticism it is that the book lacks details of adequate testing for, although the author states that the book must be read in conjunction with his *Laboratory Manual of Fruit and Vegetable Products*, that Manual is more for the training of the technologist in understanding the processes of preparing fruit and vegetable products than for providing a scientific study of the processes and scientific testing. Anyone not well acquainted with the industry will find in this new book a surprising wealth of information, useful not only to the food technologist and the factory manager but also to those who have to use and examine the products. Even the experienced technologist or scientist working in these industries will find much valuable information, and moreover will find it in an exceptionally well presented and compact form; every sentence is worth its presence in the book.

The book is divided into 35 chapters, as follows—I. Micro-organisms in Relation to Fruit and Vegetable Products. II. General Principles and Methods. III. Brief History of Canning. IV. Tin and Glass Containers. V. General Considerations in Establishing a Cannery. VI. Washing, Blanching, and Peeling Fruits and Vegetables. VII. Grading Fruits and Vegetables for Canning. VIII. Syrups and Brines Used in Canning. IX. Exhaust and Vacuum. X. Processing of Canned Fruits and Vegetables. XI. Canning of Fruits. XII. Pickling and Canning of Olives. XIII. Canning of Vegetables. XIV. Spoiling of Canned Foods. XV. Unfermented Fruit Beverages. XVI. Fruit and Vegetable Syrups and Concentrates. XVII. Pectin, Jellies, and Marmalades. XVIII. Fruit Jams, Butters, Preserves, and Confections. XIX. Tomato Products. XX. Sun Drying of Fruits. XXI. Dehydration of Fruits. XXII. Dehydration of Vegetables. XXIII. Packing of Dried Fruits and Vegetables. XXIV. Vinegar Manufacture. XXV. Pickles. XXVI. Olive and Coconut Oils. XXVII. Utilisation of Waste Fruits and Vegetables and Disposal of Wastes. XXVIII. Citrus By-products. XXIX. Packing Cases and Other Packages. XXX. Wines. XXXI. Frozen-Pack Fruits and Vegetables. XXXII. Vitamins. XXXIII. Plant Pigments and Related Compounds. XXXIV. Enzymes of Fruits and Vegetables. XXXV. Plant Sanitation.

The chapters that desirably might have been fuller are the last five. Of these, XXXI describes a new industry, rapidly progressing, and of which therefore a description can never be really up to date, and chapters XXXII, XXXIII and XXXIV are, of course, designed for the technologist, not for the specialist in these subjects; chapter XXXV has already been referred to.

The book is well printed and singularly free from printing errors. It is recommended to any chemist or technolgist or factory manager concerned with the food industry. E. B. Hughes.

Proteins and Amino-acids in Nutrition. Edited by Melville Sahyun, M.A., Ph.D. Pp. xvi + 566. New York: Reinhold Publishing Corporation. 1948. Price 45s.

This books contains a number of articles on discrete aspects of the proteins and essential amino acids together with an extensive appendix on the composition of American foods.

The individual essays are, on the whole, competent and comprehensive (up to 1946–47). There are four chapters that deal thoroughly with modern views on the use of protein in the treatment of a wide variety of clinical conditions. Of particular interest are the very large amounts of protein that appear to be required in certain circumstances, *e.g.*, after fractures of bones or extensive burns, and the correspondingly enormous amounts of other nutrients that must also be given to ensure the proper utilisation of this protein. The metabolism of protein and the relations between protein metabolism and the vitamins and hormones are well discussed, but a freer use of structural formulae would have clarified much of the interesting paper by Deuel.

There are chapters on the economics of food proteins and nutritive aspects of meat and meat products, but little is said about methods of protein and amino-acid analysis. Nowhere is there any good account of the techniques that may be used for assessing biological value or digestibility, although Mitchell contributes a paper in which current methods are reviewed. Chemical methods of amino acid analysis are not even mentioned.

It is difficult to find any reason why a chapter on filtrable viruses and another on the protein nature of toxins, antitoxins and related substances should be included in a book entitled "*The Proteins and Essential Amino-Acids in Nutrition.*" The space could have been much better used for a good introductory chapter indicating recent trends in the field or to deal more fully with topics such as digestion or analytical methods, which are but briefly mentioned.

This book is one of a type that is becoming increasingly familiar; it is a collection of reviews written by different workers, none of whom attempts to cover more than a fraction of the subject matter. In such publications the editor's responsibility is to ensure that the subject is reasonably well covered and that the articles bear a direct relation to the subject as indicated in the title. This book, like many others of its type, would have been much improved if the separate parts had been more thoroughly integrated.

W. F. J. Cuthbertson

PHYSICAL METHODS GROUP

A meeting of the Group, at which the subject will be "Electrophoretic Analyses," will be held at 3.30 and 5.30 p.m. on Friday, April 1st, in the Chemistry Lecture Theatre of The University, Nottingham. The following papers will be read:

3.35 p.m. "The technique of moving boundary electrophoresis," by R. A. Kekwick, D.Sc.

4.05 p.m. "Electrophoresis in the analysis of serum proteins," by N. H. Martin, M.A., M.B., M.R.C.P., F.R.I.C.

5.30 p.m. "The effect of Antrypol on the electrophoretic pattern of sera," by A. E. Ambler, B.Sc., Ph.D., and J. Madinaveitia, Ph.D.

5.30 p.m. "Ionophoresis of amino acids and peptides," by A. J. P. Martin, M.A., Ph.D.

Members of the Biochemical Society are invited to attend the meeting.

SECOND INTERNATIONAL CONGRESS OF CROP PROTECTION

The Second International Congress of Crop Protection, under the presidency of the Rt. Hon. The Viscount Bledisloe, P.C., G.C.M.G., K.B.E., F.S.A., will be held in London from the 21st to the 28th of July, 1949.

It will be organised in six Sections as follows—Section 1, Insecticides; Section 2, Fungicides; Section 3, Plant Growth Regulators; Section 4, Toxicology of Crop Protection Substances; Section 5, Methods of Application; Section 6, Analytical Methods and Standardisation.

The charge for membership is £2 0s. 0d. sterling. Forms of application for membership, on which particulars as to Sections in which it is desired to take part and papers it is desired to read are invited, may be obtained from the Honorary Organiser, Lt.-Col. Francis J. Griffin, Second International Congress of Crop Protection, 54, Victoria Street, London, S.W.1.

FEBRUARY, 1949 Vol. 74, No. 875

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin

Part I

The Determination of Benzyl Penicillin by Precipitation with N-Ethylpiperidine

INTRODUCTION

AT a meeting held at the Ministry of Health in December, 1946, under the chairmanship of Sir Weldon Dalrymple-Champneys, the importance of obtaining agreement on standard methods of determining the various penicillins was stressed. Accordingly, another meeting was convened on February 10th, 1947, by the Ministry of Health. This meeting, under the chairmanship of Dr. A. A. Miles of the National Institute for Medical Research, was attended by representatives of the various manufacturers of penicillin, the Ministry of Supply, the Ministry of Health and the National Institute for Medical Research, and was known as the "Conference on the Differential Assay of Penicillin." The practicability of the methods of differential assay was discussed in the light of recent experience and it was decided to examine further the N-ethylpiperidine precipitation method for the determination of benzyl penicillin. The results obtained by members of the Conference, after examination of some twelve samples of penicillin by their own interpretations of the method, showed such wide variation that, at a meeting of the Conference held on June 17th, 1947, it was decided to appoint a sub-committee of analysts from the four manufacturing firms and the Ministry of Supply (Chemical Inspectorate), with the specific assignments of (1) examining and assessing the validity of the N-ethylpiperidine precipitation method, with a view to standardising and improving it if possible, so as to render it a suitable method for the determination of benzyl penicillin in samples of penicillin containing approximately 90 per cent. or more of benzyl penicillin and (2) setting up and evaluating a working standard penicillin containing approximately 90 per cent. of benzyl penicillin and 10 per cent. of other penicillins. The evaluation of this standard was to be made by comparison with a purified sample of crystalline benzyl penicillin containing 98 to 99 per cent. of benzyl penicillin (subsequently referred to as the "A.S.C. Standard"), which was in turn to be compared with the purest sample of benzyl penicillin available to the Conference (subsequently referred to as the "M.U. Standard" or Manufacturers Ultimate Standard). With the increasing purity of commercial penicillin it had become difficult to obtain from ordinary stocks samples containing only 90 per cent. of the benzyl compound; it was decided, therefore, at later meetings of the Conference that a salt containing a higher proportion of benzyl penicillin would be acceptable as a working standard.

The Sub-Committee appointed was as follows—

C. R. Bond, M.Sc., F.R.I.C., Imperial Chemical Industries Ltd., (*Chairman*).
L. J. Bellamy, B.Sc., Ph.D., Ministry of Supply
A. R. Graham, Ph.D., A.R.I.C., Distillers Company Ltd. (from October, 1947)
T. Henshall, M.A., D.Phil., Distillers Company Ltd. (until October, 1947)
T. E. V. Horsley, B.Sc., F.R.I.C., Glaxo Laboratories Ltd.
G. F. Hall, M.B.E., B.Sc., F.R.I.C., Boots Pure Drug Co. Ltd., (*Hon. Secretary*).

and, at some meetings, it has been pleased to receive the assistance of

P. D. Coppock, M.Sc., F.R.I.C., Distillers Company Ltd.
A. F. Lerrigo, B.Sc., F.R.I.C., Glaxo Laboratories Ltd.
M. E. D. Windridge, M.A., B.Sc., F.R.I.C., Robinson Bros. Ltd.

The method under consideration was proposed by Sheehan, Mader and Cram[1] and was subsequently adopted, with modifications, by the Food and Drug Administration of the United States. It consisted, briefly, of extraction of an acidified aqueous solution of the penicillin salt with amyl acetate and subsequent precipitation of the N-ethylpiperidinium salt from a mixture of amyl acetate and acetone. The Sub-Committee, after consideration of the various modifications in use, agreed upon the following procedure.

METHOD

REAGENTS—

In the course of the investigation it became apparent that, to obtain satisfactory results, the reagents must be of a high standard of purity. In particular, the use of highly purified N-ethylpiperidine is essential. It is hoped that, as a result of discussion with the manufacturers, a suitable grade of N-ethylpiperidine will be generally available.

Special Note—All reagents shall be cooled in an ice water bath before use.

I. *Acetone solution*—Acetone (A.R.), containing not more than 0·3 per cent. of water, saturated with the N-ethylpiperidinium salt of benzyl penicillin at room temperature, cooled in ice-water and gravity-filtered.

The water content of the acetone is determined by the method of Smith and Bryant[2] modified as follows: (*a*) The solution is left with occasional swirling for at least 10 minutes after the addition of the first portion of absolute alcohol. (*b*) α-Naphtholphthalein is used as the indicator. The best end-point is obtained by adding a small excess of alkali and titrating back with 0·2 *N* acid after addition of fresh indicator.

II. *Amyl acetate solution*—Amyl acetate (A.R.) saturated with the N-ethylpiperidinium salt of benzyl penicillin at room temperature, cooled in ice-water and gravity-filtered.

III. *Acetone - amyl acetate solution*—A mixture of equal volumes of acetone and amyl acetate, both of the standard of purity described above, saturated with the N-ethylpiperidinium salt of benzyl penicillin at room temperature, cooled in ice-water and gravity-filtered.

IV. *Phosphoric acid solution*—2 ml. of phosphoric acid B.P., diluted to 10 ml. with distilled water.

V. *N-ethylpiperidine solution*—2 ml. of N-ethylpiperidine diluted with 8 ml. of amyl acetate (A.R.), saturated with the N-ethylpiperidinium salt of benzyl penicillin at room temperature, cooled in ice-water and gravity-filtered.

The N-ethylpiperidine used shall comply with the following specification.

Purity (from alkalinity to bromophenol blue):	99·5 to 100·5 per cent.
Boiling range:	95 per cent. shall distil between 129·5° and 131·5° C. and the distillation shall be complete at this temperature.
Refractive index (20° C.):	1·4438 to 1·4440.
Specific gravity	
(15·5°/15·5° C.):	0·827 to 0·829.
(20°/20° C.):	0·825 to 0·827.
Storage test:	Store under air in diffused daylight at 20° C. for 3 days; no change in colour and no precipitation shall occur.

Carbon disulphide test: Mix 0·5 ml. of the sample with 5 ml. of light petroleum (50° to 60° C.). The solution shall be clear and colourless. On the further addition of 1 ml. of carbon disulphide the solution shall be clear and colourless.

Test for piperidine: It shall contain not more than 0·4 per cent. of piperidine when tested by the following method. Transfer 1 ml. to a test tube and add, shaking vigorously after each addition, 1 ml. of freshly distilled carbon disulphide, 5 ml. of 20 per cent. w/v copper sulphate solution, 10 ml. of chloroform and, from a burette, 25 per cent. v/v sulphuric acid drop by drop until the blue-green gelatinous precipitate has redissolved and both layers are clear. Compare the colour of the lower layer by transmitted light with that of blank tests carried out in precisely the same manner, in which 0·2 ml., 0·4 ml., 0·6 ml., 0·8 ml. and 1·0 ml. respectively of 0·5 per cent. w/v aqueous piperidine solution are taken in place of the N-ethylpiperidine.

Appearance: It shall be free from turbidity and shall be practically colourless. Samples not complying with this requirement shall be redistilled.

VI. *Sodium sulphate*—Powdered anhydrous sodium sulphate (A.R.) that has been freshly ignited.

PROCEDURE—

Special Note—After the initial weighing of the sample it is essential that all operations shall be carried out under ice-cold conditions.

From a suitable stoppered weighing bottle (Note 1) weigh accurately, by difference, about 120 mg. of the sample (Note 2), into a 15-ml. centrifuge tube. Add 5 ml. of water and dissolve. Stand the tube in ice-cold water and allow to cool. Add 5 ml., accurately measured, of amyl acetate solution (Reagent II) and 0·5 ml. of phosphoric acid solution (Reagent IV); stopper with a wet cork and shake immediately for 15 seconds. Centrifuge for 30 seconds. Pipette off the aqueous layer completely, add to the centrifuge tube 0·5 g. of sodium sulphate (Reagent VI), stir, allow to stand for 5 minutes in ice-cold water and then centrifuge for 30 seconds. Replace in ice-cold water for 5 minutes, pipette off a 3-ml. aliquot into a suitable tared centrifuge tube (Note 3) containing a thin glass rod slightly curved at the end. Add 3 ml. of acetone solution (Reagent I) and 1·5 ml. of N-ethylpiperidine solution (Reagent V); stir, close the mouth of the tube with a rubber thimble, place the tube inside a test tube, close the test tube with a cork and stand it in ice-cold water for 2 hours (Note 4). Remove the centrifuge tube and centrifuge for 1 minute. Break the surface to dislodge any particles at or above the surface of the liquid and again centrifuge for 1 minute. Decant off the supernatant liquor and wash the precipitate by stirring with 2 ml. of acetone - amyl acetate solution (Reagent III) followed by centrifugation for 1½ minutes and decantation. Wash twice by stirring with 2 ml. of ether (Note 5) followed by centrifugation for 1½ minutes and decantation. Smear the deposit over the inside of the tube by means of the curved glass rod and dry at room temperature *in vacuo* to constant weight, the tube being placed in a nearly horizontal position.

$$\text{Per cent. sodium benzyl penicillin by weight} = \frac{\text{weight of precipitate}}{\text{weight taken}} \times 132{\cdot}8$$

N.B.—The determination of total penicillins, which is required for the expression of results as a percentage on total penicillins, should be made by the iodimetric procedure described in Part II of this Report.

Notes—

(1) A weighing bottle of small diameter is advisable. Its weight should be not more than 15 g.

(2) With 100,000-unit packed bottles, freeze-dried *in situ*, the contents of two bottles should be taken as completely as possible.

(3) The total weight of the centrifuge tube and the glass rod should be not more than 15 g.

(4) A standing time of 2 hours was adopted since higher recovery and greater uniformity of results were thereby obtained than at ½ or 16 hours.

(5) As the solubility of the precipitate, especially in acetone, is considerably influenced by rise of temperature, it was decided to adopt the washing procedure detailed above.

The main difficulty confronting the Sub-Committee in its work was the determination of the absolute recovery by the method in the absence of a sample of benzyl penicillin the purity of which could be proved by any known means. It was therefore decided to accept the M.U. Standard as 100 per cent. sodium benzyl penicillin, because available methods did not indicate measurable amounts of impurities in it and it was the most highly purified sample of benzyl penicillin examined by the collaborators.

The results of determinations on the M.U. and A.S.C. Standards are given below; micro-chromatograms[3] and infra-red spectra were examined and the potency was determined by repeated microbiological assay. The various results by individual workers were in close agreement.

M.U. STANDARD

Other penicillins—

X (by micro-chromatogram)	trace;	(by infra red spectra)	Not detectable within limits of accuracy of method (less than 2 per cent.)
F ” ”	nil ;	” ” ” ”	
D ” ”	nil ;	” ” ” ”	
K ” ”	nil ;	” ” ” ”	

Potency (biological assay): 1710 units/mg. (±60 units)
(British National Standard ≡ 1600 units/mg.).

Sodium benzyl penicillin by method described:	Member	A	98·2 per cent.
	”	A	98·6 ”
	”	B	99·0 ”
	”	B	99·0 ”
	”	B	98·8 ”
	”	B	98·4 ”

A.S.C. STANDARD

Other penicillins—

X (by micro-chromatogram)	$<$ 0·1%;	(by infra red spectra)	Not detectable within limits of accuracy of method (less than 2 per cent.)
F ” ”	*ca.* 0·3%;	” ” ” ”	
D ” ”	$\leqslant$0·1%;	” ” ” ”	
K ” ”	$\leqslant$0·1%;	” ” ” ”	

Potency (biological assay) (mean figures): (British National Standard ≡ 1600 units/mg.)	Member	A	1643 units/mg.
	”	B	1681 ”
	”	C	1678 ”
	”	D	1636 ”
	”	E	1683 ”
Sodium benzyl penicillin by method described (mean figures):	Member	A	95·4 per cent.
	”	B	96·9 ”
	”	C	95·0 ”
	”	D	96·2 ”
	”	E	96·6 ”
Grand mean:			96·1 ” (S.E. 0·19)

Assuming the purity of the M.U. Standard to be 100 per cent., the Sub-Committee is of the opinion that, at this level, a 98·6 per cent. recovery figure is obtained. Accordingly, as the (weighted) mean assay result for the A.S.C. Standard is 96·1 per cent., after correction on a 98·6 per cent. recovery basis, it has been assigned a sodium benzyl penicillin content of 97·5 per cent.

In order to examine further the reproducibility of the method, determinations were also made on an N-ethylpiperidinium salt of penicillin; the following results were obtained—

N-ETHYLPIPERIDINIUM SALT OF PENICILLIN

Benzyl penicillin by method described (calculated as N-ethylpiperidinium benzyl penicillin) (mean figures):	Member A	97·5 per cent.
	» B	96·6 »
	» C	95·3 »
	» D	95·7 »
	» E	97·5 »
Grand mean:		96·5 per cent.

Statistical examination of the individual determinations on this sample, which were heavily weighted by twenty-six determinations by member C, showed the weighted mean to be 96·1 per cent. (S.E. = 0·16) and also showed that the differences between laboratories were significant.

In order to complete the work on the examination of the validity of the proposed method the composition of a range of samples was determined by employing iodimetric assay to determine the approximate amount of inert matter, and micro-chromatographic assay to determine what proportion of the penicillins was benzyl penicillin. The composition of the N-ethylpiperidine precipitates was also examined micro-chromatographically, so as to determine to what extent co-precipitation with other penicillins was taking place, and iodimetrically to determine the amount of inert material in the precipitate (Table I).

In all these micro-chromatographic examinations the method of calculation given by Goodall and Levi[3] was employed, using, where available, more up-to-date figures for the *B. subtilis* potencies of the various penicillins in the conversion from percentage composition in *B. subtilis* units to percentage composition by weight. In view of the considerable error to which the micro-chromatographic method is subject it must be borne in mind that all the values quoted for the composition of samples and precipitates can only be approximate.

One of these samples (PPD/AS/0239—an N-ethylpiperidinium salt) had a composition approximating more nearly than any other available sample to the limiting level of 90 per cent. benzyl penicillin at which it was proposed to use the method. This was examined in more detail than the other samples with the results shown below.

SAMPLE PPD/AS/0239

Benzyl penicillin determined by the method described (calculated as N-ethylpiperidinium benzyl penicillin) (mean figures):	Member A	93·1 per cent.	
	» B	92·8 »	
	» C	92·6 »	
	» D	91·8 »	
	» E	93·5 »	
Grand mean:		92·7 »	(S.E. 0·36)

The differences between laboratories were not significant.

Proportions of penicillins in the sample and in its N-ethylpiperidinium precipitate, by micro-chromatographic analysis.

	Laboratory B		Laboratory C	
Penicillin	Sample	Precipitate	Sample	Precipitate
X	0·1	0·1	0·1	0·1
U_1	0·5	0·5	0·3	0·4
G	93·3	93·6	96·1	97·4
F	2·9	2·4	1·3	1·3
D	2·3	2·3	1·3	0·8
K	1·1	1·0	0·8	—
No. of determinations:	4	2	4	3
G range:	92·2 to 93·9	93·4 to 93·7	95·9 to 96·4	96·4 to 98·3
Potency (*S. aureus*) u./mg.	1342	—	1340	1370

TABLE I

Sample	Potency *S. aureus* units	Penicillin % (iodim.) w/w	Benzyl penicillin as % of total penicillins (micro-chromatogram) *B. subt.* units		Benzyl penicillin % in sample $\frac{(b) \times (d)}{100}$	Benzyl penicillin by pptn. % (uncorrected)	Benzyl penicillin as % on total penicillins in precipitate (micro-chromatogram) *B. subt.* units		Penicillin in precipitate (iodim.) % w/w (of theoretical)
			units	w/w			units	w/w	
	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)
M.U. Standard	1710	100	99·9	99·9	99·9	98·6	99·9	99·9	—
A.S.C. Standard	1664	98·5	99·7	99·5	98·0	96·1	98·8	97·6	100·2
48/0239 ..	1340	100	96	93	93	92·8	97	94	
48/0058	1490	87	72	56	49	47·1	90	83	99·2
365	660	37·7	81	69	26	13·3	93	88	87·0
48/0372	—	95·7	93	83	84	81·9	96	92	99·9
48/0373		95·3	92	86	82	83·0	94	90	99·8
2269/29/3	—	100	9	5	5	0·0	—	—	—

Note (1). Sample 48/0239 is an N-ethylpiperidinium salt, and figures under (*b*) and (*f*) are calculated accordingly.

Note (2). Sample 2269/29/3 was a synthetic mixture of penicillin sodium salts virtually free from inactive impurities composed as follows.

Penicillin	X	0·2 per cent. w/w	Penicillin	F	31·2 per cent. w/w
"	U_1	0·2 "	"	D	45·5 "
"	G	4·7 "	"	K	18·0 "

Note (3). As a basis for calculation of the iodimetric figures, a mean molecular weight of 356 (*i.e.*, the molecular weight of sodium benzyl penicillin) has been used throughout. With penicillin containing 90 per cent. of G or over, with which this work is primarily concerned, the errors thereby introduced are slight; even if the sample contained 90 per cent. of G and 10 per cent. of F, where the maximum error would be met, the G figure would be incorrect only to the extent of 0·66 per cent.

Note (4). As a basis for calculation, the following conversion factors (*B. subtilis* units) for the individual penicillins were used.

X	1500	Schmidt, Ward and Coghill[4]
U_1	1500	Assumed—no data available—traces only present
G	1667	By definition
F	970	Schmidt, Ward and Coghill[4]
D	660	Determined on pure specimens prepared by T. Leigh (I.C.I.). (National British Standard ≡ 1600 u./mg.)
K	845	

Note (5). The nomenclature used for the various penicillins in the order of the zones on the chromatographic strip is as follows.

Penicillin	X	*p*-Hydroxybenzyl penicillin
"	U_1	—
"	G	Benzyl penicillin
"	F	Pent-2-enyl penicillin
"	D	*n*-Amyl penicillin
"	U_2	—
"	U_3	—
"	U_4	—
"	K	*n*-Heptyl penicillin
"	U_5	—

A study of the results given in these tables shows that the precipitation procedure gives a precipitate which, except with the already pure M.U. Standard, is not composed of the N-ethylpiperidinium salt of pure benzyl penicillin (compare (*c*) with (*g*) and (*d*) with (*h*) in Table I). It is shown however in Table I that, in the range 90 to 100 per cent. benzyl penicillin, there is some agreement between the calculated benzyl penicillin content of the samples examined and the amount determined by the proposed method. It may well be, therefore, that there is, in fact, a compensation of errors in this range.

The standard deviation of some thirty determinations on the A.S.C. Standard and thirty-one determinations on the N-ethylpiperidinium salt was 1·24 per cent.

In view of these findings the Sub-Committee recommends that the procedure described be used as a tentative method in the examination of samples of penicillin consisting substantially of benzyl penicillin. In consideration of the appreciable error to which the proposed method is subject and its susceptibility to slight variations in the quality of the reagents used, to temperature, etc., and inasmuch as the differences in the results on the same sample between laboratories were sometimes significant, the Sub-Committee considers it is desirable that a standard penicillin should be assayed alongside the test sample and a correction applied equal to the difference between the benzyl penicillin content found for this standard and its actual value. For reference purposes the Sub-Committee, with the approval of the Ministry of Health, has standardised a quantity of sodium benzyl penicillin, designated the "Benzyl Penicillin Gravimetric Standard," to which a value of 95·8 per cent. sodium benzyl penicillin has been assigned.

The Sub-Committee is indebted to all those who have assisted in the experimental work, to Imperial Chemical Industries Ltd., for providing the M.U. and A.S.C. Standards, to the Distillers Company Ltd. for providing the sample of N-ethylpiperidinium salt, to Mr. Primavesi of the Distillers Company for his modification of the Smith and Bryant method, to Robinson Bros. Ltd. for their gift of N-ethylpiperidine and to Mr. Windridge for permission to include the details of the test for piperidine.

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January, 1949

Ether Peroxide as a possible Source of Error in the Röse-Gottlieb Butter-Fat Test

BY M. M. MUERS AND MISS M. A. HOUSE

(*Read at the meeting of the Society on Wednesday, October 6th, 1948*)

IN the course of control work in the manufacture of evaporated milk, it was observed that the figures for fat reported by a country creamery control laboratory were nearly always slightly higher than the Central Laboratory figures for the duplicate samples. Both laboratories used the Mojonnier method, and each obtained agreement to within 0·05 per cent. of fat between its own duplicate tests, but the differences between the means for the two laboratories ranged from 0·02 to 0·19 on a fat figure of 9 per cent. The blank for both sets of reagents was negligible, but the use of creamery reagents at the Central Laboratory confirmed local results. Subsequently, similar differences were found for two other creameries.

Ethyl ether, used in the analysis, is known to form peroxide and it seemed possible that this might react with butter fat and thereby increase its weight. Such an effect has been found by Middleton[1] with oleic acid; and, since this work was started, a similar suggestion has been put forward by Janse,[2] but without any experimental evidence. As samples of the ether used at the three creameries all gave strong peroxide reactions, the above suggestion was tested, with the results shown in Table I.

TABLE I

FAT IN EVAPORATED MILK, MOJONNIER METHOD

Ether from	Other solvents from	Laboratory	Fat found per cent.
Creamery (peroxide-positive)	Creamery	Creamery	9·39, 9·38
" "	"	Central	9·41, 9·41
Central Laboratory (peroxide-negative)	"	"	9·31, 9·32
" " "	Central Laboratory	"	9·34, 9·36

In analyses by the Röse - Gottlieb and Werner - Schmid methods similar or larger differences were encountered. Also, when mixtures of a known weight of pure butter-fat with specially purified solvents, in the proportions used for the Röse - Gottlieb analysis, were evaporated and heated to constant weight in the usual manner, the following results were obtained (means of duplicates).

TABLE II

Control (no solvents)	99·48% recovery
All pure solvents	99·76% "
Peroxide-positive ether, other solvents pure	100·8% "

These results show that the major part of the effect is in fact due to ether containing peroxide.

Owing to the supply position in war-time, large stocks had been held at the country laboratories, where some of the ether was several years old, whilst Central Laboratory ether was fresh and peroxide-free. Peroxide estimations on the old ether by the method of Nozaki[3] gave amounts of active oxygen up to 0·08 per cent., and if this all combined with the fat it would be ample to account for the observed differences.

In order to prevent the discrepancies and possible risk of explosion which may arise from use of peroxidised ether, it was desired to treat it in such a way that peroxide would be removed and prevented from reappearing under normal laboratory conditions. For use in factory laboratories, a simple method which would avoid such operations as distillation or filtration of large volumes of ether was necessary. Additions of small amounts of many antioxidants have been suggested in the literature, *e.g.*, diphenylamine, β-naphthol or hydroquinone. Of those tried, only diphenylamine had any effect, but it was unsuitable because it caused rapid discoloration. The use of various metals has also been reported, but Bailey and Roy,[4] using ether dried over sodium, found that only sodium amalgam was really effective in preventing peroxide formation in daylight. It has now been found that many metallic couples, especially zinc - copper, magnesium - copper, iron - tin and aluminium - mercury completely remove peroxide from *moist* ether and prevent its re-formation even in bright daylight. The presence of a little water is necessary except with aluminium - mercury, which has a slow action even in dried ether. Fifty-ml. lots of peroxidised ether, dried over anhydrous calcium sulphate to a water content (Karl Fischer titration) of 0·01 per cent., were treated with 100 sq. cm. of metal surface, alone and in presence of 2 per cent. of added water, and titrated at intervals as shown in Table III.

TABLE III

REMOVAL OF PEROXIDE FROM ETHER BY VARIOUS COUPLES

The figures represent ml. of 0·02 *N* thiosulphate per 5 ml. of ether

Duration of treatment	Al - Hg	Fe - Cu	Fe - Sn	Mg - Cu	Zn - Cu	Control
Without added water:						
Days 0	—	—	—	—	—	13·6
1	2·8	12·6	12·5	12·6	12·8	13·6
" 5	1·8	11·6	13·2	12·7	13·0	13·6
With 2% of added water						
Days 0	—	—	—	—	—	13·5
" 1	0·0	0·2	0·5	0·4	0·3	13·9
" 2	—	—	0·1	0·2	0·0	—
" 5	0·0	0·0	0·0	0·0	0·0	14·2

The reaction is evidently a reduction of peroxide with formation of metal hydroxide. Aluminium and magnesium couples cause an inconvenient effervescence in moist ether. For treatment of ether intended for fat determinations, we prefer a zinc - copper couple prepared by immersing zinc foil in acidified copper sulphate solution for about a minute, washing, and transferring it while still wet to the ether container. Satisfactory results have been obtained in 5-gallon cans, using seven or eight 50- × 5-cm. strips of the treated zinc foil. The zinc hydroxide formed is dissolved both by the ammonia used in the Röse - Gottlieb and by the hydrochloric acid used in the Werner - Schmid method, and therefore the treated ether need not be filtered. Such ether gives a negative test for peroxide with titanic acid reagent, and gives results for fat analyses agreeing very closely with those obtained with pure re-distilled ether, as shown by the examples cited in Table IV.

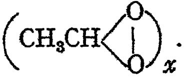
TABLE IV

FAT ESTIMATIONS IN MILK PRODUCTS

The figures are means of duplicates

	Pure ether %	Peroxide-positive ether %	Treated ether %
Evap. milk, Röse - Gottlieb	9·28	9·48	9·34
" "	9·03	9·09	9·01
Butter-fat "	100·4	101·0	100·3
Milk powder, Werner - Schmid ..	26·66	26·97	26·70

Further investigation of the reaction between butter-fat and ether peroxide has provided some information on the fate of the peroxide oxygen. Pure butter-fat was treated by the procedure for a normal Röse - Gottlieb fat estimation, using purified reagents, except for peroxidised ether, and the amount of peroxide was determined at each stage. It was found that 13 per cent. of the original peroxide was washed out into the aqueous phase during the extraction stage, and 5 per cent. passed over into the distillate during evaporation of the solvents. The residual vapour and solvent in the flask, removed as usual at this stage by a gentle and brief current of air, was highly peroxidic, accounting for 77 per cent. of the total. The 5 per cent. of the peroxide remaining in the fat fell to 4 per cent. after heating to constant weight. Since the amount of active oxygen in this 5 per cent. would, by itself, account for an increase in weight of only 0·4 per cent., compared with up to 2 per cent. found in fat analyses, it seems that some other constituent of the peroxidised ether must be involved. Possibly the whole of the ether peroxide molecule combines with or is retained by the fat. The increase in weight of pure butter-fat treated with peroxidised ether alone was roughly proportional to the amount of peroxide present, and did not seem to depend on the amount of fat. Peroxidised ether itself left no weighable residue on heating, but when large amounts of peroxide were present with the fat, the latter took very long, up to 6 hours, to reach constant weight. This suggested that part of the weight increase might be due to a loose association, or possibly to a purely physical effect of the fat in delaying evaporation of some less volatile constituent of the ether. This theory was supported by the fact that the peroxidic distillate from a fat determination, when added to a further sample of fat, again caused a weight increase, though less than in the first determination. Moreover, purified B.P. paraffin, which would not be expected to react chemically with peroxide, showed a small increase in weight when treated with peroxidised ether. The destruction of the yellow colour of butter-fat, presumably by oxidation, during evaporation with ether peroxide, could not account for the weight increase because this was unaffected by preliminary removal of the colouring matter with charcoal.

It has been reported by Rieche[5,6] that "ether peroxide" contains chiefly a polymeric ethylidene peroxide  $\left(CH_3CH\langle{}^{O}_{O}\rangle\right)_x$. A small quantity of this substance was prepared by his method, but without isolating the intermediate or final products from ether solution, in order to minimise risk of explosion. The product, diluted with pure ether to a peroxide content comparable with that of stored ether, had properties qualitatively similar to the latter in nearly all respects, but it was less volatile in solvent vapour and caused a much larger (5 per cent.) increase in weight of butter-fat. The intermediate product, hydroxyethyl hydroperoxide, and also ethereal hydrogen peroxide (both of which were probable impurities in our preparation), caused no increase in weight. Treatment of the ethylidene peroxide solution with a zinc - copper couple removed all peroxide and eliminated nearly all the weight increase effect. Although not conclusive, this evidence does indicate that part of the material estimated as peroxide in stored ether is, in fact, Rieche's substance, and causes the weight increase, whilst the bulk of the peroxide is the less reactive part which passes over with the solvent vapour in a fat determination.

SUMMARY—

1. In butter-fat analyses by the Mojonnier, Röse - Gottlieb and Werner - Schmid methods, the use of ether containing peroxide gives values that are too high by amounts up to 2 per cent. of the fat figure.

2. Peroxide can be removed, and prevented from re-forming, and correct fat values can be obtained, by treating such ether with metallic couples in presence of moisture. Zinc - copper couple is preferred, as the zinc hydroxide formed does not interfere with the analysis.
3. In a fat estimation most of the peroxide present is driven off with the solvents. The active oxygen left in the fat residue is insufficient to account by itself for the observed increase in weight of the fat.
4. The effect is roughly proportional to the amount of peroxide present.
5. Even pure B.P. paraffin shows a slight increase in weight when treated with peroxidised ether, although the latter alone leaves no non-volatile residue.
6. An ethereal solution of ethylidene peroxide has most of the properties of "ether peroxide," except that it causes a much larger increase in weight.
7. The results indicate that ethylidene peroxide is probably the constituent of "ether peroxide" chiefly responsible for the weight increase effect, but that some other component besides the active oxygen must be retained by the fat.

Our thanks are due to the Directors of United Dairies Limited, for permission to publish this paper.

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UNITED DAIRIES LIMITED
CENTRAL LABORATORY
WOOD LANE, LONDON, W.12

April, 1948

DISCUSSION

The PRESIDENT asked if there was any danger of an explosion with 0·08 per cent. of active oxygen in the ether and, if not, at what concentration the danger point was reached.

Dr. K. A. WILLIAMS said that explosions had occurred in the removal of ether from solutions of oxidised and polymerised oil eluted from chromatographic columns of alumina, and that these explosions had caused some damage. It was found that they were due to the use of ether that had been stored for some time, and it might well be that the danger of explosion was enhanced by the presence of the oxidised oil. He had for many years made it a practice in his own laboratory never to use ether without first distilling it from solid caustic soda. This method of purification seemed to remove peroxides satisfactorily, and, when it was used, he had never had any difficulty from them.

Dr. E. C. WOOD said that although distillation from caustic soda removed the peroxide it did not prevent its re-formation, as did the authors' method of adding a strip of zinc - copper couple to the stock bottle.

Mr. J. M. JONES asked if any work had been done with other ethers. *iso*Propyl ether seemed to form peroxides more readily than diethyl ether; moreover, the distillation residues from *iso*propyl ether exploded more regularly than did those from diethyl ether.

Mr. A. L. BACHARACH asked whether the recorded increases in weight of butter-fat might not be the result of atmospheric oxidation catalysed by peroxidised constituents of the ether. If so, one would expect the increases to exceed the unaccounted-for portion of the etherial peroxides.

Mr. C. B. CASSON said that in his experience the fat extracted by peroxidised ether was not completely soluble in light petroleum, which suggested that polymerisation had taken place. He had found that the increased weight of the fat could be prevented by washing the extract with an acid sulphite solution before evaporation.

Mr. N. HERON said that the suggestion made by Mr. Bacharach that the oxidation of the oil is catalytic was probably true. The nature of the oil or fat has an effect on the extent of the reaction. Recently, during the determination of linseed oil in paints, an explosion occurred just at the end of the distillation of the ether. The ether used was from a general laboratory stock and no similar trouble was experienced in the extraction of fats of a more saturated nature, *e.g.*, milk-fat or beef stearin.

He had also observed other abnormal oxidations of vegetable oils. Sunflower seed oil, after drying at 100° C. yielded a large amount of material insoluble in light petroleum. The total ether extract of a sample recently examined was 12 per cent., but only 8 per cent. was soluble in petrol. The residue was also insoluble in methylated ether.

Dr. MUERS, in reply, said that from the literature it is certain that quite small amounts of peroxide may cause explosion, but for some very fortunate reason (possibly the presence of butter-fat), there have been no explosions during this work, even when deliberate attempts were made to produce one, on a small scale, by heat or shock.

Distillation over sodium hydroxide is one of many known methods for removing ether peroxide, but such methods are unsuitable for use with large volumes of ether by inexperienced workers in a factory laboratory. The authors' method is preferable because it is safe and simple. The treated ether gave negative tests for peroxide with vanadic acid, titanic acid, and potassium iodide - acetic acid reagents. No work has been done with *iso*propyl ether.

It is possible that part of the weight increase under discussion is due to atmospheric oxidation of the fat in the oven. The small amount of peroxide in the residue may be the remains of the ether peroxide left after evaporation, or fresh peroxide formed during heating. But the residue does reach a minimum weight during heating, and this stage is delayed when highly peroxidised ether is used, which is the reverse of what would be expected if peroxide catalysed fat oxidation.

The heated butter-fat residue from an extraction with peroxidised ether is quite soluble in light petroleum. In fact, in a fat determination the final net weight of fat is found by dissolving out the residue with petroleum ether and re-weighing the dry empty flask; but drying oils would no doubt behave differently.

Quantitative Photometric Determination of Diphenyl in Orange Peel

By A. P. STEYN and F. ROSSELET

EXTENDED use of wrappers impregnated with diphenyl for preserving citrus fruits has necessitated a search for an analytical method of sufficient sensitivity to assay the diphenyl absorbed by the fruit from the wrappers. The colorimetric method used by Tomkins and Isherwood[1] lacks sensitivity and, in consequence, Cox[2] suggested a photometric method based on the absorption characteristics of diphenyl dissolved in *cyclo*hexane. Cox, however, did not develop the method for the problem under discussion. The results presented in this paper describe the development of an absorptiophotometric method of determining diphenyl in the micro-quantities present in citrus fruits that have been preserved by diphenyl-impregnated wrappers.

To indicate the quantities involved it may be mentioned that a medium-sized orange weighing 200 g. requires a wrapper 10 × 10 inches. Such a wrapper impregnated at the rate of 40 mg. per 100 square inches would contain 40 mg. of diphenyl. The greater part of the diphenyl absorbed by the fruit is found in the peel of the orange, so the maximum proportion that would normally be expected in the peel of a medium-sized orange is of the order of 650 p.p.m. Owing to losses of diphenyl by volatilisation to the air, the maximum concentration indicated above is seldom attained in practice. In order to correlate diphenyl content and preservative action the analytical method should be able to determine 0·2 p.p.m. of diphenyl with an accuracy to within 5 per cent.

ABSORPTION CHARACTERISTICS OF DIPHENYL—

Since diphenyl has a well-marked absorption band in the ultra-violet, this affords a simple and accurate method for its estimation.[2] According to Cox, diphenyl in *cyclo*hexane shows an absorption maximum at 251 mμ. having an $E^{1\%}_{1\,cm.}$ value of 1200. A solution containing 0·001 per cent. of diphenyl will give a convenient reading in a 1-cm. cell.

The absorption curve of 20 p.p.m. of diphenyl in *cyclo*hexane determined within the range 230 to 400 mμ. is presented in Fig. 1. Diphenyl shows maximum absorption at 247 mμ. with an $E^{1\%}_{1\,cm.}$ value of approximately 1100. These differences in peak and extinction values from those obtained by Cox may be due to the respective purity of the samples of diphenyl used, or to changes in the optical system.

The purity of the diphenyl (B.D.H. brand) used in this laboratory was compared with spectrochemically pure diphenyl (Merck's brand). Both have absorption peaks at the same wavelength and the same $E^{1\%}_{1\,cm.}$ value. To eliminate the optical factor, density readings are taken not at 247 mμ., but at 250 mμ., because the density is constant between 249 and 251 mμ.

Spectrochemically pure chloroform cannot be used as a solvent,[1,2] because it absorbs light at and below 250 mμ. Spectrochemically pure *cyclo*hexane is the most suitable solvent since (*a*) the solubility of diphenyl as well as orange oil in *cyclo*hexane is high, and (*b*) *cyclo*hexane does not absorb light at and below 250 mμ. The method for the purification of *cyclo*hexane is based on work done by Allsopp and Twyman.[3]

In the absence of a spectrophotometer and as a first approach, the absorption characteristics of standard solutions of diphenyl in spectrochemically pure *cyclo*hexane ranging in concentration between 0·6 and 20 p.p.m. were determined on a Hilger medium quartz spectrograph, using nickel electrodes and a 1-cm. cell. The variations in the logarithm of the relative intensity of the line Ni 2501 recorded on an A.R.L. comparator-densitometer are plotted in Fig. 2 against the logarithms of the concentrations and give a straight line relationship. The accuracy is, however, only to within about 10 per cent. and is not considered sufficiently sensitive.

Through the courtesy of the University of Cape Town, the use of a Beckman quartz spectrophotometer, Model DU, as described by Cary and Beckman,[4] was made available. The spectrophotometer is more accurate and more convenient than the spectrograph for absorption work and the mean percentage error of the determination of diphenyl in the standard solutions was of the order of 2·2 per cent.

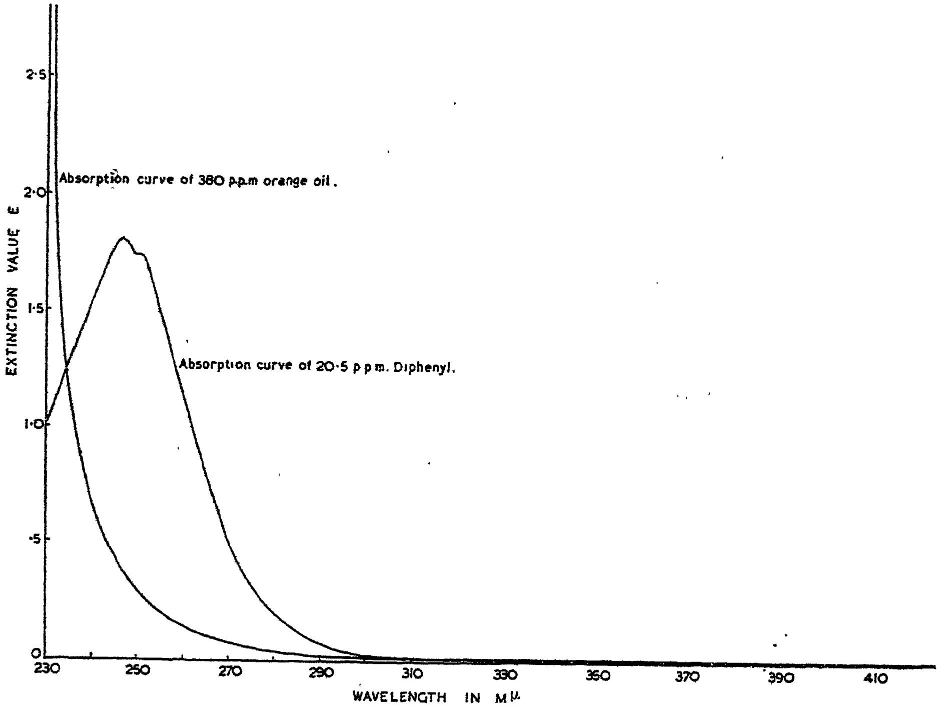

Fig. 1. Absorption curves of 20 p.p.m. diphenyl and 380 p.p.m. orange oil in *cyclo*hexane.

EXTRACTION OF DIPHENYL FROM ORANGE PEEL—

The system of extraction used by Tomkins and Isherwood has proved very satisfactory. The peel is minced and steam-distilled and the distillate extracted with *cyclo*hexane. Since the solubility of diphenyl in water is very low—less than 1 p.p.m.—three successive extractions of the distillate with *cyclo*hexane suffice to remove all but traces of diphenyl. Unfortunately, the orange oil present in the distillate is also extracted by the *cyclo*hexane. Orange oil interferes in both the colorimetric method and the absorptiometric method of estimating diphenyl. In the following sections methods of eliminating the interfering effects of orange oil are presented.

THE ELIMINATION OF INTERFERING SUBSTANCES—

When the distillate from 300 g. of minced peel is extracted successively with three 50-ml. portions of *cyclo*hexane and the extracts are combined, the solvent normally contains between 3 and 4 per cent. of orange oil, irrespective of the amount of diphenyl present.

Tomkins and Isherwood used successive treatments with concentrated sulphuric acid to destroy the orange oil. Their results were subject to an error of about 20 per cent. It was found in the course of the present work that not only did the sulphuric acid destroy the orange oil, but it also affected the diphenyl recovery values. This effect is considerable, as indicated in the following tables.

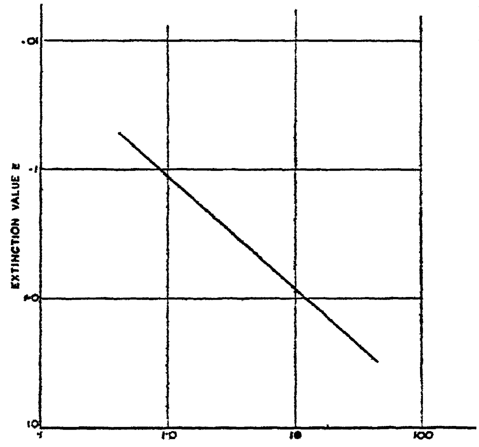

Fig. 2. Working curve for diphenyl in *cyclo*hexane.

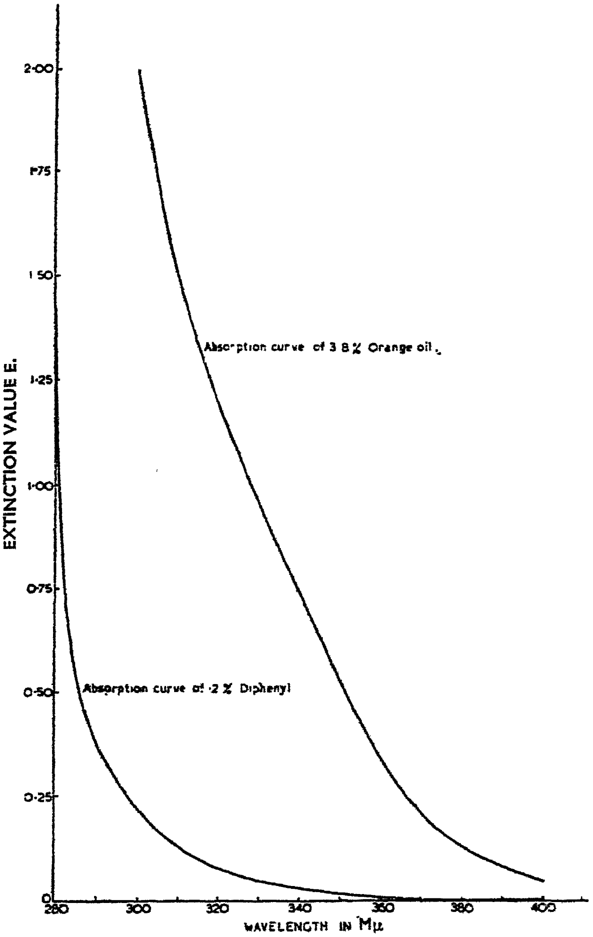

Fig. 3. Absorption curves of 3·8 per cent. orange oil and 0·2 per cent. diphenyl in *cyclo*hexane.

TABLE I

EFFECT OF CONCENTRATED SULPHURIC ACID TREATMENT OF A MIXTURE OF DIPHENYL AND ORANGE OIL IN *cyclo*HEXANE ON DIPHENYL ESTIMATION

Treatment of sample	Mean recovered p.p.m.	Error %
82·2 p.p.m. diphenyl	82·0	−0·24
82·2 p.p.m. diphenyl washed with 2 × 75 ml. of distilled water	82·5	+0·36
82·2 p.p.m. treated with 4 × 20 ml. concentrated sulphuric acid and washed with water	66·6	−19·0
82·2 p.p.m. + 1 ml. of Navel orange oil treated with 4 × 20 ml. sulphuric acid and washed with water	55·3	−32·8
82·2 p.p.m. + 2 ml. of Navel orange oil treated with 4 × 20 ml. sulphuric acid and washed with water	48·3	−41·2

The percentage error increases with every increase in orange oil content.

TABLE II

RECOVERY OF DIPHENYL AFTER CONCENTRATED SULPHURIC ACID TREATMENT OF THE EXTRACT OF THE STEAM-DISTILLATE OF MINCED ORANGE PEEL TO WHICH KNOWN QUANTITIES OF ORANGE OIL AND DIPHENYL HAD BEEN ADDED

No. of oranges	Orange oil added ml.	Diphenyl added to orange oil mg.	Diphenyl in *cyclo*hexane p.p.m.	Recovery value diphenyl p.p.m.	Error %
5	2	16	137·01	93·0	− 32·1
5	1	8	68·5	43·8	− 36·0
5	0·5	4	34·25	27·0	− 21·6

The removal of orange oil from the extract of the steam-distillate by repeated treatments with concentrated sulphuric acid results in considerable losses of diphenyl as well. In view of these results it is probable that the large errors experienced by Tomkins and Isherwood in determining diphenyl by their colorimetric method arose from the successive treatments of the *cyclo*hexane extracts with concentrated sulphuric acid.

CORRECTION FOR ABSORPTION BY ORANGE OIL—

In view of the difficulties of removing orange oil from the extracts, the question arises whether the errors introduced in the absorptiometric method by the presence of orange oil can be eliminated by determining the absorption by orange oil at a wavelength where diphenyl shows little or no absorption, and whether a correction can be applied for the absorption of the orange oil when determining diphenyl at 250 mμ.

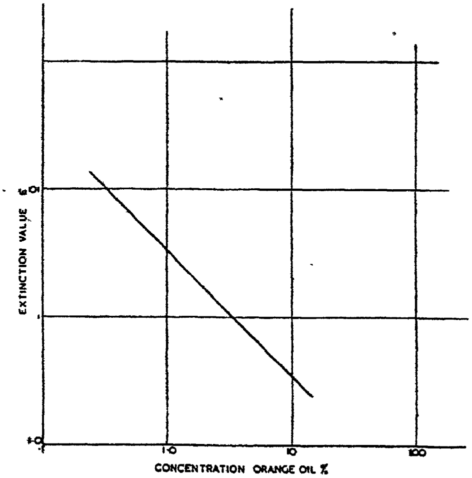

Fig. 4. Working curve for orange oil.

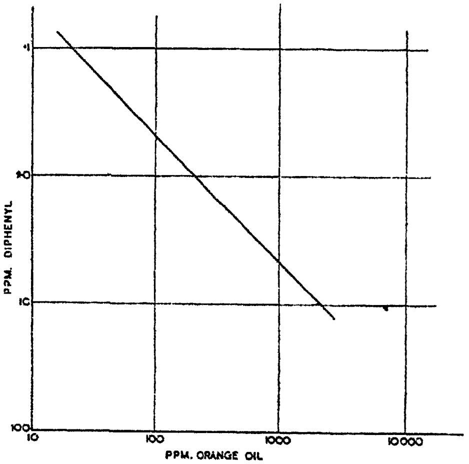

Fig. 5. Curve of relationship between diphenyl and orange oil at 250 mμ.

In the study of the absorption characteristics of orange oil a sample of oil from Navel oranges was prepared by steam-distillation, and this sample has served as the standard throughout. The absorption characteristics of this oil have not changed during storage for a period of 14 months. All the investigations to date have been confined to Navel oranges, and hence the complicating factor of differences in the oil characteristics due to variety and species is eliminated from the present investigations. Such difficulties will no doubt be found important and necessitate separate calibration curves for different varieties and species of citrus fruits. Experience alone will indicate whether fruit of the same variety grown in different districts will show sufficient differences to call for separate calibration curves.

Fig. 3 indicates the absorption curve between 300 and 400 mμ. of a 3·8 per cent. solution of the "standard" Navel orange oil in *cyclo*hexane. On the same graph the absorption curve for a 0·2 per cent. diphenyl solution in *cyclo*hexane is also indicated. Since diphenyl shows little or no absorption above 350 mμ., the possibility of using the region above 350 mμ. for assaying orange oil in presence of diphenyl is evident. The wavelength selected for this purpose is 375 mμ. The E values at 375 mμ. for a range of orange oil concentrations between 0·1 and 10 per cent. are represented by a straight line, see Fig. 4.

Since the extract of the steam-distillate has to be diluted 100-fold for the determination of the absorption of diphenyl at 250 mμ., the absorption curves between 230 to 400 mμ. of a 0·002 per cent. diphenyl solution and of a 0·038 per cent. orange oil solution are presented in Fig. 1. The absorption of diphenyl in the ultra-violet band is clearly defined by the peak at 247 mμ. Orange oil, however, has no peak at or near the diphenyl peak, but the absorption increases rapidly at the shorter wavelengths.

In order to determine the quantities of diphenyl and orange oil giving equal absorption at 250 mμ., the absorption of standard concentrations of orange oil ranging from 0·005 to 0·04 per cent. were determined at 250 mμ. These values were converted into unit diphenyl values from the calibration curve for diphenyl. The orange oil and diphenyl equivalents thus obtained, when plotted on log-paper, show a linear relationship, Fig. 5.

Provided there is no chemical interaction between the orange oil and diphenyl, the absorption at 250 mμ. will be the same as the constituent absorptions of diphenyl and orange oil at that wavelength, since

$$d = 1\,(\epsilon_1 C_1 + \epsilon_2 C_2 + \ldots)$$

where C_1, C_2 . . . are the concentrations of the absorbing substances having molecular extinction coefficients of ϵ_1, ϵ_2, ...[5]

Since the total absorption of mixtures of diphenyl and orange oil in *cyclo*hexane agrees very closely with the sum of their separate absorptions, as indicated in Table III, it can be assumed that there is no chemical reaction between the diphenyl and the orange oil, and hence the method is applicable.

TABLE III

Concentrations (a) of orange oil %	E value 250 mμ.	Concentrations (b) of diphenyl %	E value at 250 mμ.	E value of samples of (a) + (b) at 250 mμ.	Sum of E values of (a) and (b)	Error %
0·03	0·165	0·000257	0·260	0·405	0·425	+ 5·0
0·04	0·215	0·001028	0·900	1·080	1·115	+ 3·1
0·05	0·260	0·000514	0·490	0·740	0·750	+ 1·35
					Mean	3·1%

METHOD ADOPTED

The method adopted for the determination of diphenyl in the presence of orange oil is as follows:—

(*a*) Determine the E value of the undiluted *cyclo*hexane extract of the steam-distillate of the sample at 375 mμ. and convert into concentration of orange oil from the graph, Fig. 4.

(*b*) Convert the value of orange oil obtained in (*a*) above into the equivalent value of diphenyl by means of the graph, Fig. 6.

(*c*) Dilute the *cyclo*hexane extract 100-fold. Determine the E value at 250 mμ. and convert into concentration of diphenyl from the graph, Fig. 2.

(*d*) Subtract the value obtained in (*b*) from the value obtained in (*c*) to obtain the actual amount of diphenyl present.

The accuracy of the method may be gauged from the following data.

Table IV gives the results of an analysis of fifteen mixtures of diphenyl and orange oil analysed by the method adopted, together with the percentage error of each determination.

Known concentrations of diphenyl dissolved in orange oil were made up and added to the minced peel of five test oranges. The distillate, recovered from the steam-distillation, was extracted in *cyclo*hexane and the concentration of diphenyl determined. Table V gives the data of this analysis.

Known concentrations of diphenyl dissolved in *cyclo*hexane were made up and added to the minced peel of five test oranges, the distillate was extracted, and the concentration of diphenyl determined.

The accuracy of the method depends mainly on the purity of the *cyclo*hexane. Benzene present in the sample does not have an appreciable effect on optical density readings taken at 375 mμ., but has a large effect on readings taken at 250 mμ.

TABLE IV

ANALYSIS OF FIFTEEN MIXTURES OF DIPHENYL AND ORANGE OIL WITH ERRORS OF EACH DETERMINATION

Contents of sample	E value at 375 $m\mu$. (a)	Diphenyl equivalent of orange oil (b)	E value at 250 $m\mu$. (d)	Diphenyl equivalent of (d) in p.p.m. (c)	True diphenyl in p.p.m. $(c-b) \times 100$	Error %
0·1028% diphenyl						
+5% orange oil ..	0·182	2·58	1·128	13·0	10·42	+ 1·36
+4% ,, ..	0·142	2·00	1·068	12·3	10·30	+ 0·19
+3% ,, ..	0·106	1·50	1·041	11·9	10·40	+ 1·17
0·0514% diphenyl						
+5% orange oil ..	0·178	2·50	0·705	7·72	5·22	+ 1·36
+4% ,, ..	0·142	2·00	0·660	7·04	5·05	− 1·95
+3% ,, ..	0·105	1·48	0·620	6·60	5·12	− 0·26
0·0257% diphenyl						
+5% orange oil ..	0·176	2·45	0·480	4·90	2·45	− 2·14
+4% ,, ..	0·143	2·05	0·455	4·55	2·50	− 2·70
+3% ,, ..	0·104	1·45	0·408	4·02	2·63	+ 2·34
0·0128% diphenyl						
+5% orange oil ..	0·178	2·50	0·385	3·75	1·25	− 2·34
+4% ,, ..	0·142	2·00	0·350	3·38	1·38	+ 7·80
+3% ,, ..	0·108	1·58	0·302	2·85	1·27	− 0·78
0·0064% diphenyl						
+5% orange oil ..	0·176	2·45	0·328	3·05	0·60	− 6·24
+4% ,, ..	0·144	2·08	0·293	2·73	0·65	+ 1·56
+3% ,, ..	0·107	1·55	0·241	2·17	0·62	− 3·12
					Mean ..	2·3%

TABLE V

RECOVERY VALUES OF DIPHENYL DISSOLVED IN ORANGE OIL ADDED TO MINCED PEEL

Orange oil added ml.	Diphenyl added to peel %	Diphenyl recovered %	Error %
2	0·013701	0·01400	+ 2·2
1	0·006850	0·00690	+ 0·7
0·5	0·003425	0·0035	+ 2·2
		Mean	1·7%

TABLE VI

RECOVERY VALUES OF DIPHENYL DISSOLVED IN *cyclo*HEXANE ADDED TO MINCED PEEL

Diphenyl added to minced peel %	Diphenyl recovered %	Error %
0·01619	0·0160	− 1·2
0·01563	0·0155	− 0·8
0·00757	0·0076	+ 0·4
0·00405	0·00395	− 2·5
	Mean	1·2%

The absorption cells must be rinsed with spectrochemically pure *cyclo*hexane after every reading, because a drop containing 0·001 per cent. of diphenyl, left over in the cell, will cause an error of 10 per cent. when a sample of 0·0001 per cent. solution of diphenyl is placed in the cell.

The mean percentage error in adopting this method for the determination of diphenyl in presence of orange oil is 2 per cent.

The method is sensitive enough to determine as little as 0·00002 per cent. (0·2 p.p.m.) of diphenyl in a sample.

SUMMARY

The colorimetric method for the estimation of diphenyl in orange peel as adopted by Tomkins and Isherwood is not sensitive enough to correlate diphenyl content with its preserving action, since the percentage error is of the order of 20 per cent. A more accurate method is desired. The procedure for the extraction of diphenyl from the orange peel is based on their work, but for the actual determination of diphenyl another method had to be evolved.

A spectrophotometric method was suggested by Cox, using *cyclo*hexane as a solvent for the determination of diphenyl at 251 mμ. The present work shows that when diphenyl is dissolved in *cyclo*hexane and the absorption curve is determined between 230 and 400 mμ., the maximum absorption lies at 247 mμ. with an $E^{1\%}_{1\,\mathrm{cm.}}$ value of 1100. Since the absorption of diphenyl is constant between 249 and 251 mμ., working curve values are taken at 250 mμ. The maximum concentration of diphenyl that can be determined is 20 p.p.m. For higher concentrations suitable dilutions can be made.

To eliminate the interfering effect of orange oil in the absorption of diphenyl at 250 mμ., sulphuric acid was used to destroy it. The use of sulphuric acid, however, results in too low diphenyl values.

The following method was evolved for the determination of diphenyl in presence of orange oil.

Since orange oil shows absorption between 280 and 400 mμ. and diphenyl no absorption above 350 mμ., the concentration of orange oil can be determined by its absorption at 375 mμ. in the undiluted *cyclo*hexane extract. The absorption at 250 mμ. after diluting 100-fold indicates the concentration of diphenyl plus orange oil; the concentration of the latter being obtained from the linear relationship between diphenyl and orange oil from the extraction of the latter at 375 mμ.

Recovery tests as carried out show a mean percentage error of 2 per cent.

We wish to thank the Director of the Low Temperature Research Laboratory, Cape Town, Mr. Rees Davies, for valuable criticism and guidance; Mr. A. G. Visser for the drawing and Mr. D. J. de Villiers for the photographing and printing of the figures, and Dr. H. M. Schwartz of the C.S.I.R., Chemistry Department, University of Cape Town, for the use of their Beckman photometer.

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CAPE TOWN, SOUTH AFRICA

May, 1948

Analysis of Rare Earth Oxides by means of Emission Spectra

Part I. Persistent Lines in Arc Spectra of Rare Earth Elements

BY D. M. SMITH AND G. M. WIGGINS

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THE analysis of rare earth oxides by means of their arc emission spectra presents a number of difficulties on account of the great complexity of these spectra. This is particularly evident when an examination is made for other rare earth elements occurring as impurities. This paper and the one following record the results of investigations, carried out over a period of some two years, on the following problems—

1. The empirical determination of the most persistent lines of rare earth elements, for a given type of arc excitation. A preliminary note on this subject was published in this journal in 1946.[1]

2. The identification of faint lines coinciding with impurity lines.
3. Methods for avoiding the masking effects due to the presence of cyanogen bands in graphite and carbon arc spectra. (A method, using a carbon arc in steam, giving practically complete elimination of these bands is described in Part II.)

ARC EXCITATION SOURCES—

At the time these investigations were started the only satisfactory arc source available in the Company's laboratory was an intermittent A.C. arc, a low-voltage A.C. arc triggered by means of a superimposed high-frequency low-energy spark occurring at the voltage peak of the A.C. wave (modified from B.S. 1225 : 1945). Since this type of arc had proved suitable in other cases of trace analysis, an arc between pure graphite electrodes, taking 7 amp., was first used for the analysis of rare earth oxides.

Subsequently two D.C. arc sources became available, and these were tested in comparison with the intermittent A.C. arc. One was a simple D.C. arc circuit, 100-volt open circuit voltage, obtained by half-wave rectification of A.C., smoothed with a suitable choke. The other was the Hilger constant-current D.C. arc source, the current strength being 5·6 amp. The arc gap in each case was 3 mm.

EMPIRICAL DETERMINATION OF THE MOST PERSISTENT LINES OF THE RARE EARTH ELEMENTS

It was concluded from a study of the available data on persistent lines of the rare earth elements[2,3,4,5] that the empirical determination of the most persistent lines, under the conditions of arc excitation used for the analysis of rare earth oxides, was a necessary preliminary investigation.[1]

This was done by photographing successive exposures of a continuously burning arc until the whole of the material had been volatilised, using as electrode materials both pure graphite and pure silver rods. This investigation was mainly carried out with the intermittent A.C. arc. Also, drops of dilute chloride solutions evaporated on the electrodes have been used to give faint spectra of the rare earth elements, showing only the principal sensitive lines. The latter method was also used to provide superposed (overlapping) comparison spectra to assist in the recognition of impurity lines (and coincidences) in the spectra of the rare earth oxides (see Fig. 3*b*).

A Hilger Fully Automatic Littrow spectrograph was used to record the spectra, and was set for the region 2960 to 6000 A., since according to Meggers[6] the most persistent lines were expected to be found in this wavelength region. Ilford Special Rapid Panchromatic plates were used, these being sensitive over the whole range studied and also suitable in other respects. In subsequent tests a wider range of spectrum was examined, using photographic plates with appropriate emulsions.

The results obtained are given below in Table I and, for comparison, hitherto unpublished results by Meggers and Scribner, of the National Bureau of Standards, Washington,* are also included. The latter results were derived from similar observations to determine the strongest lines of 70 elements, including rare earths, excited in a 220-v., 10-amp. D.C. arc between copper electrodes, containing one or more of the elements in atomic ratios of 1000 to 1. The authors are indebted to Dr. Meggers for kindly making these results available, thus providing an authoritative check on their own observations.

Whilst there is a considerable measure of agreement between the two sets of data, occasionally there are marked discrepancies, *e.g.*, in the assignment of the most persistent line of neodymium. As suggested by Meggers,* this may be due to differences in excitation sources. According to Gerlach and Reidl[2] the relative intensities of lines are strongly influenced by the nature of the discharge for dysprosium, erbium, terbium and thulium. Variation in the sensitivities of different plates to light of different wavelengths may also have been a contributory cause.

Some difficulties were experienced in deciding, by visual estimations of intensity, on the relative sensitivities of the lines. For example, in many instances the intensities were nearly equal at the threshold value of the plate, particularly for the less persistent lines. Moreover, some lines may have been slightly enhanced in intensity by being superimposed on the spectrum background associated with strong silver and copper lines.

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* Private communication.

Only three lines are quoted for ytterbium, as these are much stronger than the other two quoted by Meggers and Scribner.

TABLE I

THE MOST PERSISTENT LINES OF RARE EARTH ELEMENTS*

Wavelength	Intensities: Arc	Intensities: Spark	Sensitivity: M. and S. (D.C.)	Sensitivity: Authors (A.C.)	Sensitivity: Authors (D.C.)
1. CERIUM					
4186·599 II	80	25	U1	U1	U1
4137·646 II	25	12	U3	U3	U3
4133·800 II	35	8	U2	U2	U2
4040·762 II	70	5	U5	U5	U4
4012·388 I, II	60	20	U4	U4	U4
3942·151 II	35	8	—	—	U4
3801·529 II	25	3	—	—	U5

The intensities of the lines 4012·388 and 4040·762 A. are almost equal in the A.C. arc; the intensities of the last four lines are almost equal in the D.C. arc.

Wavelength	Intensities: Arc	Intensities: Spark	Sensitivity: M. and S. (D.C.)	Sensitivity: Authors (A.C.)	Sensitivity: Authors (D.C.)
2. DYSPROSIUM					
4211·719 I	200	15	U2	—	U5
4186·810 I	100 w	12	U3	—	—
4045·983 I	150	12	U4	U3	—
4000·454 II	400	300	—	—	U5
3968·395 II	300	—	U5	—	—
3944·692 II	300	150	—	U5	U4
3645·416	300	100	—	U2	U2
3531·712	100	100	U1	U1	U1
3407·80	150	9	—	U4	U3

Dy 3968·395 is masked by Ca 3968·468 in all spectra photographed in the authors' laboratory.

Dy 4211·719 is masked by Ag 4210·936 in A.C. silver arc spectra.

Dy 3531·712 is much enhanced in A.C. arc spectra as compared with D.C. arc spectra.

Wavelength	Intensities: Arc	Intensities: Spark	Sensitivity: M. and S. (D.C.)	Sensitivity: Authors (A.C.)	Sensitivity: Authors (D.C.)
3. ERBIUM					
4007·967 I	35	7	U4	U4	U3
3906·316 II	25	12	U5	U3	U3
3692·652	20	12	U2	U2	U2
3499·104	18	15	U3	U4	U3
3372·750	35	20	U1	U1	U1
4. EUROPIUM					
4205·046 II	200 R	50	U2	U2	U2
4129·737 II	150 R	50 R	U3	U3	U3
3971·99 I	1000 Rwh	—	U5	U5	U5
3930·503 II	1000 R	400 R	U4	U4	U4
3819·66 II	500 wd	500 wd	U1	U1	U1
5. GADOLINIUM					
3768·405	20	20	U4	U4	U4
3646·196	200 w	150	U5	U4	U5
3422·466	80	100	U1	U1	U1
3362·244	150	180	U2	U2	U2
3350·482	150	180	U3	U3	U3
6. HOLMIUM					
4103·84 I	400	400	U4	U4	U3
4053·92 I	400	200	U5	—*	U5
3891·02 II	200	40	U2	U2	U2
3796·73	20	40	U3	U5	U4
3456·00	60	60	U1	U1	U1
3398·98	40	60	—	U3	U5

* Ho 4053·92 is masked by Ag 4055·624 in A.C. arc spectra.

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* Wavelengths and intensities are taken from the *M.I.T. Wavelength Tables*. The notation for the sensitivity of the lines follows the same convention as in these tables, U1 denoting the most persistent line.

	Intensities		Sensitivity		
Wavelength	Arc	Spark	M. and S.	Authors	
			(D.C.)	(A.C.)	(D.C.)
7. LANTHANUM					
4086·714 II	500	500	U3	U3	U5
3988·518 II	1000	800	U2	U2	U2
3949·106 II	1000	800	U1	U1	U1
3794·773 II	400	200	U5	U5	U4
3790·822 II	400	300	U4	U4	U3

Except for La 3949·106 (U1) the above lines are of nearly the same intensity.

8. LUTECIUM					
3507·39 II	100	150	U4	U3	U3
3397·07 II	50	20 r	—	U4	—
3359·56 I	150	15	U5	U4	U4
3077·60 II	100	200	U3	U5	U5
2911·39 II	100	300	U2	U2	U2
2615·42 II	100	250	U1	U1	U1

Sensitive lines of wavelength shorter than 2960 A. were determined from spectra photographed with a medium size spectrograph (Hilger flat-field).

9. NEODYMIUM					
4303·573 II	100	40	U1	U4	U5
4156·083 II	10	20	U4	U5	U4
4109·455 II	30	30	U5	U3	U3
4061·085 II	40	30	U3	U2	U2
4012·250 II	80	40	U2	U1	U1

The first three lines (4303 to 4109) appear of almost equal intensity.

10. PRASEODYMIUM					
4225·327 II	50	40	U4	U4	U3
4222·98 II	125	40	U5	U3	U5
4179·422 II	200	40	U2	U5	U4
4100·746 II	200	50	U3	U2	U2
3908·431 II	100	60	U1	U1	U1

Except for Pr 3908·431 (U1) the above lines are of nearly the same intensity.

11. SAMARIUM					
3885·284 II	50	50	U5	U5	U5
3634·271 II	100	25	U4	U3	U3
3609·484 II	60	100	U3	U1	U2
3592·595 II	40	50	U2	U2	U1
3568·258 II	40	50	U1	U4	U4

The most sensitive lines, U1 to U4, appear with almost equal intensity.

12. TERBIUM					
3874·18 II	200	200	—	U5	*
3676·35	100	200	U3	U3	
3585·03	15	50	U2	—†	
3561·74	200	200	U4	U2	
3509·17	200	200	U1	U1	
3324·40	70	50	U5	U4	

* No sample available for test with the D.C. arc.

† No sensitive line at wavelength 3585·03 A. was detected in intermittent A.C. arc spectra.

13. THULIUM					
3848·018	400	250	U2	U1	*
3795·765	250	150	U5	—	
3717·92	100	10	U1	U3	
3462·20	250	200	U3	U2	
3425·08	200	300	U4	U4	
3362·61	250	200	—	U5	

* No sample available for test with the D.C. arc.

Note—The order of sensitivity has not been established with certainty for the thulium lines as the only samples available were very impure. Tm 3425·08 and 3362·61 A. are of almost equal intensity.

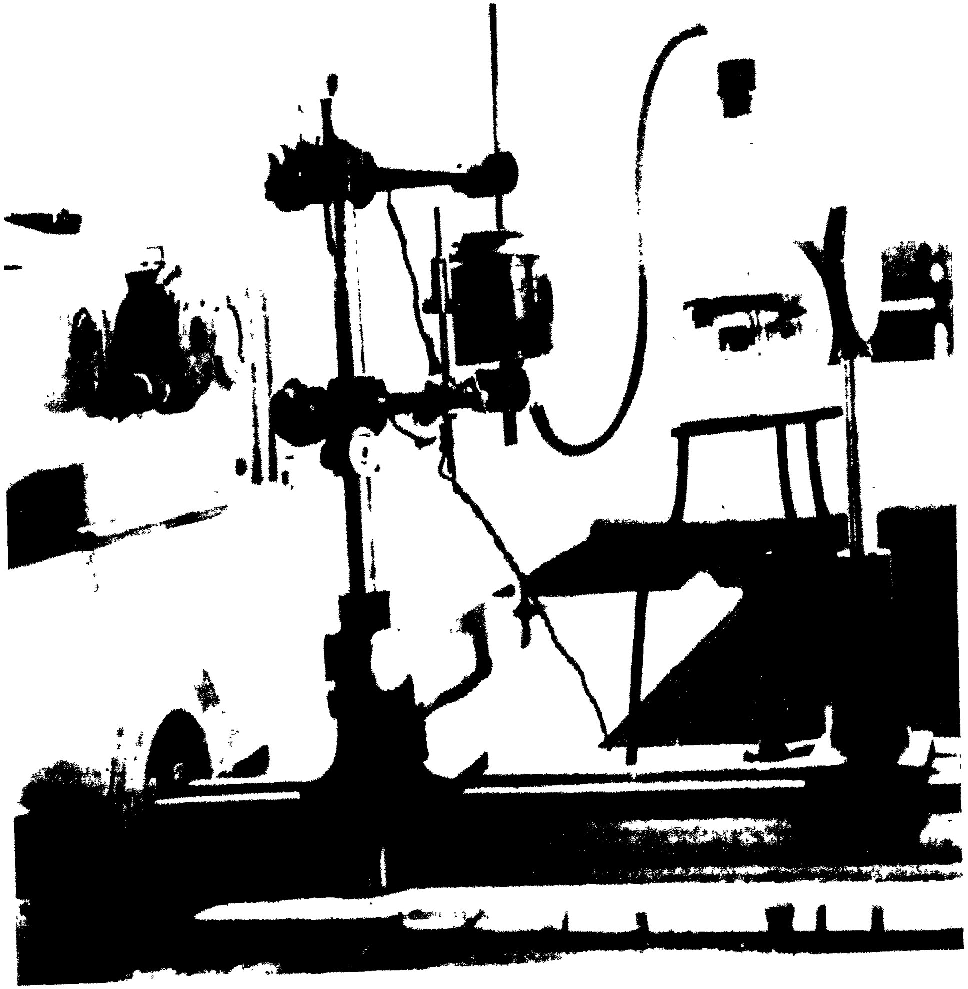
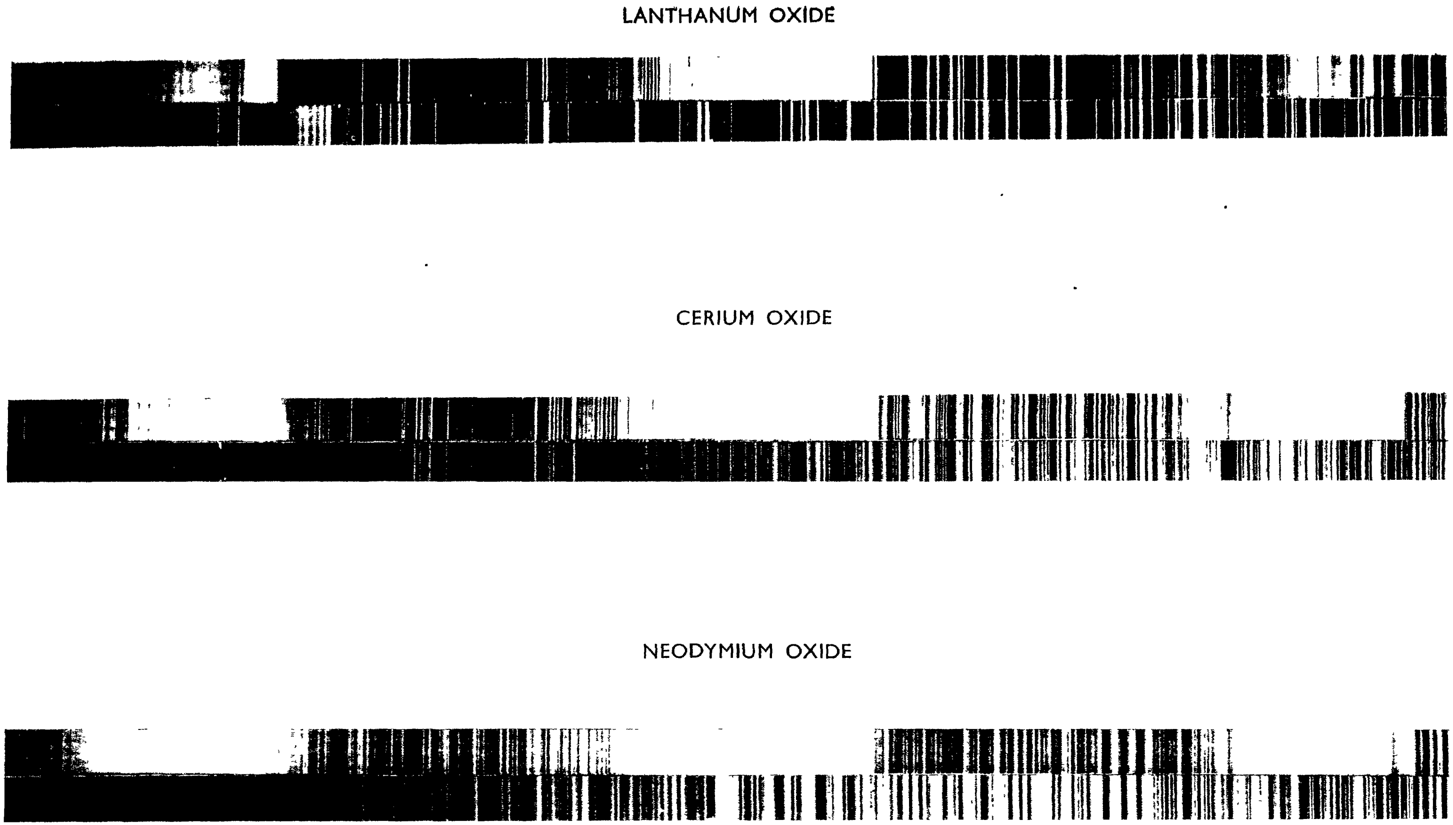


Fig. 1 Apparatus for the Carbon Arc in Steam.



LANTHANUM OXIDE

CERIUM OXIDE

NEODYMIUM OXIDE

Fig. 2. Arc spectra of rare earth oxides. The upper spectrum, in each case, is of the arc in air and the lower spectrum of the arc in steam, showing the suppression of the cyanogen bands by the latter method of excitation. (Region of spectrum: 3500 to 4230 A.)

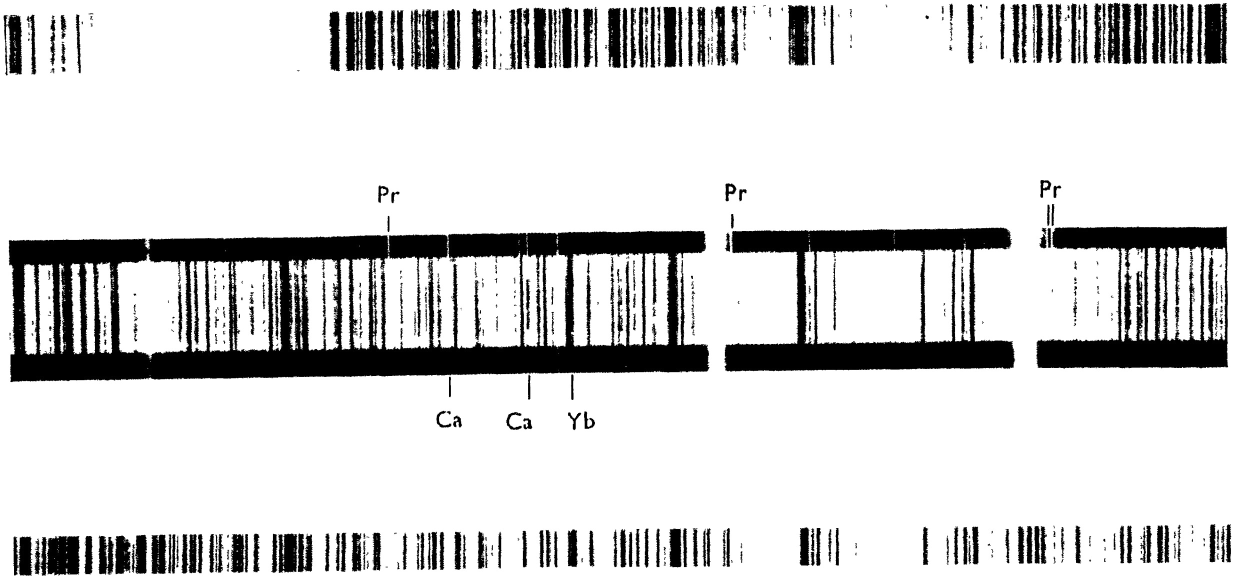
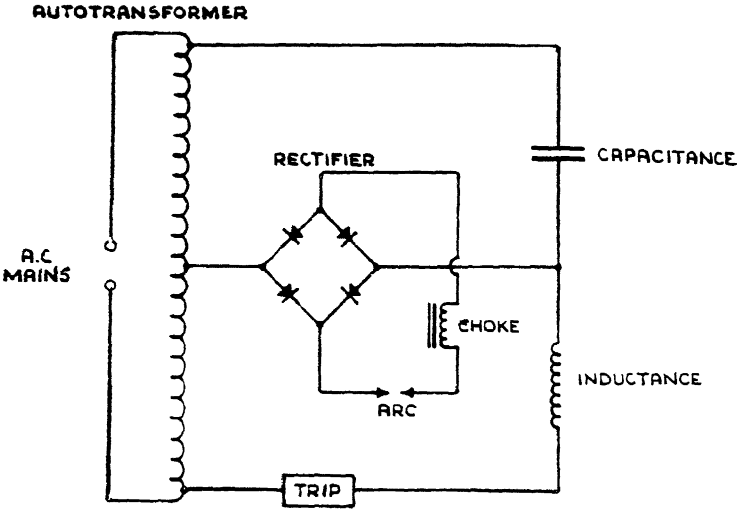


Fig. 3. Arc spectra of cerium oxide (3800–4300 A.) obtained with different electrode materials.
(*a*) Graphite arc in air.
(*b*) Silver arc in air (illustrating the use of overlapping comparison spectra).
(*c*) Carbon arc in steam.



CIRCUIT DIAGRAM OF CONSTANT CURRENT D.C. ARC

Fig. 4

TABLE I—*continued*

Wavelength	Intensities		Sensitivity		
	Arc	Spark	M. and S. (D.C.)	Authors (A.C.)	Authors (D.C.)
14. YTTERBIUM					
7699·49	2000	—	U5	—	—
5556·476 I	1500	50	U4	—	—
3987·994 I	1000 R	500 R	U3	U3	U3
3694·203 II	500 R	1000 R	U1	U2	U2
3289·37 II	500 R	1000 R	U2	U1	U1

Yb 3289·37 may be slightly enhanced by the background due to Ag 3280·683.

Wavelength	Arc	Spark	M. and S. (D.C.)	Authors (A.C.)	Authors (D.C.)
15. YTTRIUM					
4374·935 II	150	150	U2	U3	U5
4102·376 I	150	30	U5	U5	—
3774·332 II	12	100	U3	U2	U2
3710·290 II	80	150	U1	U1	U1
3600·734 II	100	300	U4	U4	U3
3242·280 II	60	100	—	U5	U4

Y 4374·935 appears as a broad diffuse line at low intensities.

IDENTIFICATION OF CERTAIN FAINT LINES IN THE SPECTRA OF RARE EARTH ELEMENTS AS BEING DUE TO OTHER RARE EARTH ELEMENTS PRESENT AS IMPURITIES

For the identification of impurity lines in rare earth spectra the standard reference book used has been the *M.I.T. Wavelength Tables*. Certain faint lines, attributed in these tables to the rare earth element under test, coincide in position with those of strong lines in the superposed comparison spectra of the impurity rare earth elements sought. Such coincidences, briefly referred to as "Significant Coincidences," are listed in Table II. The close agreement between these wavelengths and those of the strongest lines of other elements supports the conclusion that the lines in question are in fact sensitive rare earth impurity lines. In justification of this view it may be noted that in the *M.I.T. Wavelength Tables* (p. xi, "Precision of Wavelengths") it is suggested that the wavelengths of lines due to rare earths of especial rarity may not be entirely above suspicion. Moreover, the faint lines given in Table II are not recorded in the wavelength tables published by Gatterer and Junkes[5] (which were not available to the authors until the completion of the present work).

In the case of erbium, for example, the holmium line **3456·00** A. and the ytterbium line **3289·37** A. are the only lines coinciding with faint erbium lines, and these lines are shown in Table I (6, 15) as the most persistent lines of these impurity elements. The same applies to europium containing ytterbium as an impurity.

It may be noted that no impurity coincidences are listed in Table II, *infra*, for La, Lu, Tm and Yb. The data for these elements have been censored by Dr. W. F. Meggers for the *M.I.T. Wavelength Tables*.

FORTUITOUS COINCIDENCES

Further coincidences are recorded in Table III, but as the lines in the first column are assigned to the corresponding elements in the tables published by Gatterer and Junkes, these coincidences are to be regarded as fortuitous.

The data in Tables II and III are not claimed to be exhaustive lists; they record those coincident lines that will most commonly occur when the M.I.T. Tables are used for the analysis of rare earth oxides and that have been studied in connection with the present work. They are given here with the object of assisting others engaged on similar work.

METHODS FOR INCREASING THE SENSITIVITY OF DETECTION OF ONE RARE EARTH IN ANOTHER

Because the cyanogen bands in the carbon or graphite arc spectra masked a number of the persistent lines of the rare earth elements, an intermittent A.C. arc taking from 8 to 10 amp. between pure silver electrodes has been used as a routine method of analysis. The graphite arc method, however, was satisfactory for the detection of common impurities in rare earth oxides.

Table II

(Tentative identification of certain recorded faint lines as sensitive impurity lines)

Significant coincidences (M.I.T. wavelength tables)

Recorded faint lines			Coincident sensitive lines			
	Intensity			Intensity		
Element and wavelength	Arc	Spark	Element and wavelength	Arc	Spark	Sensitivity
Cerium						
3995·752	6	—	La II 3995·750	600	300	—
3988·518	8 h	—	La II 3988·518	1000	800	U2
3987·990	5	—	Yb I 3987·994	1000 R	500 R	U3
3219·948	2	—	Tb 3219·95	50	50	—
Dysprosium						
4040·76	6	—	Ce II 4040·762	70	5	U5
3634·27	3	3 h	Sm II 3634·271	100	25	U3, 4
3494·78	2	—	Ho 3494·77	30	40	—
Erbium						
4086·713	8 s	—	La II 4086·714	500	500	U3
4077·970	20 s	18 s	Dy 4077·974	150 r	100	—
4061·082	5	—	Nd II 4061·085	40	30	U2, 3
4031·690	6 w	—	La II 4031·692	400	300	—
4012·253	12	—	Nd II 4012·250	80	40	U1, 2
4000·452	35	6	Dy II 4000·454	400	300	? U5
3941·515	5	—	Nd 3941·512	60	30	—
3710·290	15 wh	8 wh	Y II 3710·290	80	150	U1
3531·714	40	25	Dy 3531·712	100	100	U1
3494·494	15	9	Dy 3494·496	100	5	—
3456·003*	25 d	10 d	Ho 3456·00	60	60	U1
3289·36	25	8	Yb II 3289·37	500 R	1000 R	U1, 2
Europium						
3462·21	5	1 h	Tm 3462·20	250	200	U2, 3
3289·38	2	—	Yb II 3289·37	500 R	1000 R	U1, 2
Holmium						
4007·96	4	3	Er I 4007·967	35	7	U4
4000·45	5	5	Dy II 4000·454	400	300	? U5
3988·00	—	6	Yb I 3987·994	1000 R	500 R	U3
3898·55	6	8	Dy 3898·544	100	—	—
3692·65	10	15	Er 3692·652	20	12	U2
3645·41	8	8	La II 3645·414	100	60	—
			Dy 3645·416	300	100	? U2
3600·73	6	—	Y II 3600·734	100	300	U4
3289·38	10	20	Yb II 3289·37	500 R	1000 R	U1, 2
Neodymium						
3306·375	12	12	Sm II 3306·372	100	40	—
Samarium						
4133·797	2	—	Ce II 4133·800	35	8	U2
3819·678	10	20	Eu II 3819·66	500 wd	500 wd†	U1
Terbium						
4186·60	2	—	Ce II 4186·599	80	25	U1
4077·97	25	2	Dy 4077·974	150 r	100	—
Yttrium						
3694·20	4	—	Yb II 3694·203	500 R	1000 R	U1, 2
3372·77	15	3	Er 3372·750	35	20	U1

* Double line; one probably a faint erbium line, although no erbium line of this wavelength is recorded by Gatterer and Junkes.

† Double line.

The silver arc gave weaker spectra than either the carbon or graphite arcs. Tests were also made with pure copper electrodes, but these proved no better in this respect. It was found that moistening the oxide with a drop of nitric acid just before making the exposure resulted in stronger spectra. Even so, the method was not sufficiently sensitive for the

TABLE III

FORTUITOUS COINCIDENCES (M.I.T. WAVELENGTH TABLES)

Faint lines			Coincident sensitive lines		
	Intensity			Intensity	
Element and wavelength	Arc	Spark	Element and wavelength	Arc	Spark
DYSPROSIUM					
3456·01	40	—	Ho 3456·00	60	60
3327·88	2	—	Y II 3327·875	60	60
3242·285	4	—	Y II 3242·280	60	100
GADOLINIUM					
4222·98 II	10	—	Pr II 4222·98	125	40
EUROPIUM					
3710·29 II	20 w	1 wh	Y II 3710·290	80	150
3319·887 II	20	5	Dy 3319·887	150	9
HOLMIUM					
3319·87	6	6	Dy 3319·887	150	9
3216·67	—	8	Y II 3216·682	40	70
NEODYMIUM					
4424·343	50	50	Sm II 4424·342	300	300
3930·506	20	30	Eu II 3930·503	1000 R	400 R
3592·595 II	20	30	Sm II 3792·595	40	50
3531·712 II	6	4	Dy 3531·712	100	100
3456·004 II	4	—	Ho 3456·00	60	60
PRASEODYMIUM					
3795·765	30 d	20 d	Tm 3795·765	250	150
TERBIUM					
3455·99	8	—	Ho 3456·00	60	60
3100·51	15	8	Gd 3100·508	100	80
YTTERBIUM					
4409·34 II	6	10	Sm II 4409·337	100	100

detection of some rare earths when present as impurities. The lack of sensitivity was confirmed by failure to detect such elements in samples known to contain rare earth impurities by the variations in colour, a sensitive test in certain cases.

It was therefore concluded that the carbon arc would provide the highest sensitivity of detection if the cyanogen bands could be suppressed. The successful solution of this problem is described in Part II.

Part II. A Technique for the Suppression of Cyanogen Bands in Carbon Arc Spectra of Rare Earth Oxides

BY G. M. WIGGINS

IN the course of the work on empirical determination of the most persistent lines of the rare earth elements, reported in Part I of this paper, it was concluded that the arc (either A.C. or D.C.) between pure silver electrodes was less sensitive in detecting impurities than that between pure carbon or graphite electrodes. Since the "cyanogen" bands (the violet system due to the diatomic molecule CN) appear strongly in the region 2960 to 6000 A., where most of the sensitive lines of the rare earth elements occur, various attempts were made to suppress the emission of these bands by running the arc in a nitrogen-free atmosphere.

CARBON ARC IN STEAM

The simplest atmosphere to produce was water vapour and a simple steam chamber to surround the electrodes was constructed (see Fig. 1). A small aluminium can with a removable lid was used, with holes to admit the electrodes and to provide a steam inlet. An asbestos sheet was fitted on the lid to provide heat insulation. The steam was produced from a flask of boiling water, about 1 litre in capacity. At first the can was fitted with a quartz window, the steam escaping through the crevices in the holes admitting the electrodes. This window was later found to be superfluous, however, as the quantity of steam produced was sufficient to expel the air from the chamber completely.

A D.C. arc, giving 8 amp. at approximately 100 volts, was obtained between carbon electrodes. The rare earth sample was tamped into a cavity, 1 mm. in diameter and 5 mm. deep, drilled in the lower, negative electrode. The discharge was focused on the slit of a Littrow spectrograph by means of a concave mirror, the arc being offset 8 cm. from the optical axis at a distance of 25 cm. from the slit and the mirror 45 cm. from the slit.

Since the discharge was remarkably brilliant, Ilford Thin Film Half-tone plates were used in place of the Special Rapid Panchromatic plates previously used. The spectrograph slit width was 0·005 mm. These conditions gave maximum resolution of the spectrum lines, the relatively slow speed of the photographic emulsion being more than compensated for by the greatly increased luminosity of the arc discharge in steam as compared with the arc in air.

Three exposures were made, of 10, 20 and 30 seconds respectively, in order to detect faint impurity lines over the whole range of spectrum photographed, the shortest exposure facilitating the detection of such lines in the crowded part of the spectrum.

Fig. 2 illustrates the effective suppression of the cyanogen bands in the spectra of the oxides of lanthanum, cerium and neodymium. A comparison of spectra obtained with the use of graphite and silver electrodes (arc in air) and the carbon arc in steam is shown in Fig. 3.

MECHANISM OF THE ARC

Carbon rods of commercial purity were used for the preliminary tests. On first striking the arc an intense blue discharge is obtained, characteristic of the carbon arc, and the electrodes cannot be opened to more than a millimetre or so without breaking the arc. It was observed that the rare earth sample rapidly fuses into a small globule which remains at the bottom of the deep hole. Both carbon rods burn rapidly until the cathode spot reaches the molten globule. Then the globule appears to flow over the surface of the electrode and volatilisation proceeds steadily. At this stage the arc length can be greatly increased and the arc colour changes to that characteristic of the particular oxide. The exposure is timed from the appearance of this colour.

However, subsequent tests made with high-purity carbon or graphite were unsuccessful; the globule did not flow over the electrode surface and was ejected as soon as it was touched by the cathode spot.

It was at first thought that the type of carbon was a critical factor in the successful operation of the method, particularly as the rod of commercial purity had a fine-grain, almost vitreous, structure of the fractured surface. Attempts were made to match the carbons originally used, the supply of which had become practically exhausted, and some similar material was obtained through the co-operation of Mr. B. S. Cooper, of the Research Laboratories of the General Electric Company Ltd., Wembley. This material also proved unsatisfactory.

The arc spectrum of the original carbon showed strong lines due to sodium, silicon, aluminium and iron. Tests were accordingly made on an intimate mixture of the rare earth oxide with finely divided pure silica. Admixture with pure alumina was also tried, but in both cases only intermittent volatilisation was produced. Finally, trials with addition of sodium silicate proved successful in producing the original type of globule which flowed over the electrode surface.

The method finally adopted is as follows. A small quantity of sodium silicate solution (about 2 drops of a concentrated solution, sufficient to wet the sample) is added to the rare earth oxide and evaporated to dryness, and the resulting powder carefully ground to give a homogeneous mixture. The ratio of sodium silicate to rare earth does not appear to be critical, providing that the proportion of sodium silicate is not excessive.

The prepared sample is then pressed into a cavity $\frac{1}{4}$-inch deep and of $\frac{1}{8}$-inch diameter drilled in a $\frac{1}{4}$-inch diameter carbon rod and this is made the anode. The Hilger constant-current D.C. arc source,* taking 5·6 amp., was used for the excitation of the spectra (see Fig. 4), as this type of arc automatically compensates for sudden changes in arc resistance which take place during the running of the arc, thereby maintaining a steady discharge.

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* A tuned circuit containing capacity and self-inductance developed in 1946 (see ref. 1).

On striking the arc in the atmosphere of steam the characteristic yellow sodium flame is observed and this persists for a minute or so. During this period the arc is focused on the slit (with the shutter closed). When the sodium has been completely volatilised the arc colour changes to the intense colour characteristic of the rare earth oxide under test. This rapidly becomes stable and the shutter is opened and the exposure made. For the first tests made with the original carbon rods, using the simple D.C. arc, the oxide sample was placed in a cavity in the cathode. In the final method adopted (constant-current D.C. arc) the sample was made the anode, as this gave more rapid volatilisation of the sodium, resulting in quicker stabilisation of the discharge.

The method of mixing the sample with sodium silicate bears some resemblance to that used by Foster[7] in an attempt to devise a satisfactory general system of spectrographic micro-analysis. Foster used powdered graphite impregnated with 0·2 ml. of a standard sodium silicate solution and tests with known small additions of silver, nickel and bismuth showed a high sensitivity of detection. He concluded that this sensitivity was due to the formation of a large-area anode spot, so that the whole of the sample took part in the discharge, the rate of distillation being relatively slow, thereby increasing the emission from each atom.

The mechanism of the arc used for the analysis of rare earth oxides, however, appears to be somewhat different, as an extended anode spot is not formed. (Moreover, no powdered graphite is added to the sample.) It is possible that the function of the sodium silicate is that of a flux combining with the oxide in some way to give a substance that volatilises more uniformly in the arc.

STANDARDISED TECHNIQUE

Preparation of sample—A drop of pure sodium silicate solution is added to about 10 to 20 mg. of the sample of rare earth compound (in general, the oxide); the mixture is evaporated to dryness and thoroughly mixed by grinding with an agate pestle and mortar.

Arc electrodes—Pure carbon rods ¼ inch in diameter. The powder is tamped into a cavity, ¼-inch deep and of ⅛-inch diameter, drilled in the lower, positive electrode.

Arc excitation—Constant-current D.C. arc (Hilger), taking 5·6 amp., the discharge taking place in an atmosphere of steam. (A simple D.C. arc, taking 8 amp., may be used as an alternative, but it is less convenient in operation.)

Arc gap—3 mm.

Spectrograph—Fully automatic Littrow spectrograph, set for the wavelength range 2960 to 6000 A.

Slit irradiation—The central portion of the arc discharge is focused on the slit by means of a surface-aluminised concave mirror.

Slit width—0·005 mm.

Photographic plate—Ilford Thin Film Half-tone.

Exposure times—Three exposures of 10, 20 and 30 seconds respectively, when the arc has become stabilised.

Plate development—3 minutes in I.D.2 developer, with simple dish-rocking.

SENSITIVITY OF THE METHOD

Owing to the rarity and high cost of materials it has not been possible to carry out extended systematic tests to determine the limits of spectrographic detection of one rare earth as an impurity in another. Some samples with known additions were available, however, and these in conjunction with information supplied by those skilled in the refining of rare earth compounds have given the following indications. Dysprosium, gadolinium, lanthanum, lutecium, yttrium and ytterbium may be detected at concentrations of less than 0·1 per cent., and with these elements erbium, europium and holmium may probably be placed. According to Gatterer and Junkes[8] as little as 0·0005 per cent. of europium can be detected in solutions of samarium chloride, and from 0·01 to 7 per cent. accurately determined by means of a D.C. arc, taking 3·75 amp., between pure carbon electrodes.

Cerium, neodymium, praseodymium and samarium appear to be much less sensitive and thulium has not yet been studied.

ACKNOWLEDGMENTS—

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DISCUSSION

Dr. W. F. ELVIDGE, with reference to the authors' method of eliminating cyanogen bands, which are always a nuisance in spectroscopy, asked if the authors had any experience as to whether their method using sodium silicate could be used in ordinary search for trace elements, particularly in those cases where the lines sought occur among the cyanogen bands.

Mr. D. M. SMITH, in reply, said they had only tried the mixture with sodium silicate in the detection of traces of rare earth element impurities in rare earth oxides. Specially prepared pure sodium silicate was used, but for this purpose the ordinary impurities, traces of which were no doubt present, were of no consequence. In the testing of other materials, however, such traces might vitiate the results, as it appeared to be extremely difficult to prepare spectrographically pure sodium silicate. On the other hand, there seemed to be no reason why the method should not be of fairly general application (with the customary blanks for comparison). He would suggest that it might be applied to the analysis of refractory oxides, such as those of titanium and zirconium.

Mr. J. HASLAM asked if the method for the exclusion of cyanogen bands could be applied to the direct determination of small amounts of impurities in plastic materials.

Mr. B. S. COOPER said that he had been using a method of running an arc in a special atmosphere which had some similarities with that described by Mr. Smith. In his experiments, however, they were using samples rich in silicon and the serious masking of faint lines was caused by the presence of SiO bands. Fortunately their interest was in a region of the spectrum remote from the CN bands, so they went to the other extreme, and instead of eliminating nitrogen, eliminated oxygen by using an atmosphere of nitrogen obtained from a cylinder. The enclosure round the arc was very similar to the authors'. This method proved most effective in eliminating the SiO bands and enabled microphotometer measurements to be made on faint impurity lines in the 2800 to 2400 A. region. A similar method had proved useful in dealing with samples rich in germanium.

With regard to Mr. Haslam's question as to whether the authors' method could be extended to deal with impurities in plastics, Mr. Cooper said that the silicon samples to which he had just referred were arced in the form of pellets. These were made in a hot mould from a mixture of the sample with an excess of pure graphite powder and a plastic binder. They arced quite satisfactorily, so there appeared to be no reason why the same method should not be used to determine impurities in plastics themselves.

Mr. E. VAN SOMEREN asked whether, in view of the greater dispersion available, it would be advisable or preferable to use a glass spectrograph or spectrometer with camera attachment for the analysis of rare earth compounds.

Dr. J. A. C. MCCLELLAND observed that as a large proportion of the lines used by the authors, and those used in his own work, did not occur in the visible part of the spectrum, a glass prism instrument would be quite unsuitable. The best instrument to use would be a grating spectrograph. Mr. D. M. SMITH confirmed these statements and added that the most persistent lines of some rare earth elements appeared in the ultra-violet part of the spectrum; in the case of lutecium (Lu II 2615·42 and 2911·39) the lines are far removed from the violet end of the visible spectrum.

The Determination of Copper in Nickel-bearing Steels and Cast Irons: a Photometric Method

By S. D. STEELE and L. RUSSELL

VARIOUS reagents capable of producing coloured compounds on reaction with copper in solution[1,2] have been applied to the determination of copper by photometric methods. Of these, sodium diethyldithiocarbamate has been found the most suitable for the determination of copper in ferrous alloys and is probably the one most widely used at the present time for this purpose. It is generally recognised, however, that when it is applied to the determination of copper in ferrous materials, precautions must be taken to prevent interference from iron, either by removal of that element from solution with ammonium hydroxide, or by the production and measurement of the copper diethyldithiocarbamate colour solution at a *p*H greater than 9·0.[3] Similar difficulties are associated with the presence of nickel in solution, and various suggestions for the removal of nickel interference have appeared in published reports, as for example, the preliminary separation of nickel by dimethylglyoxime,[4,5] the separation of copper by means of sodium thiosulphate or hydrogen sulphide, and the extraction of copper by dithizone and carbon tetrachloride.[2]

In the authors' laboratories, prior to the present investigation, photometric determinations of copper had been conducted in the presence of iron by using a solution of *p*H greater than 9·0, any nickel present having been removed as nickel glyoxime before formation of the final colour solution. Errors that had previously arisen owing to slight precipitation of the copper diethyldithiocarbamate complex had been eliminated by using larger quantities of gum acacia than those normally employed, and by ensuring that the solution was agitated during the addition of the carbamate reagent. Occasionally, however, considerable difficulty was encountered in obtaining complete removal of nickel from solution, residual quantities of nickel glyoxime precipitating after addition of gum acacia and before the absorption readings had been taken. Further, the presence of dimethylglyoxime frequently caused a brown colour in the test solution that contributed towards erroneous results.

Preliminary experiments failed to provide a satisfactory method for complete precipitation of nickel from solution by means of dimethylglyoxime without deviating considerably from the conditions necessary for the determination of copper. These experiments, however, showed that the foreign brown colour was most probably due to the presence of ferrous dimethylglyoxime complex, the production of which was traced to the action of ultra-violet radiation on solutions containing dimethylglyoxime and ferric citrate. Consequently, the extent of interference from this source was liable to vary with time and with the place in which the test solution had been allowed to stand in the laboratory. The addition of dimethylglyoxime to the blank solution to compensate for the effect of the foreign colour provided a greater degree of accuracy than previously obtained, but did not produce sufficiently satisfactory results.

In view of the unsatisfactory nature of the dimethylglyoxime separation of nickel it was decided that, for the determination of copper in nickel-bearing steels and cast irons, an alternative procedure was necessary. Rather than investigate the use of alternative reagents that might provide a more complete separation of nickel from copper, however, it was considered advisable to endeavour to modify the diethyldithiocarbamate procedure in such a manner as to permit copper determinations to be conducted in the presence of nickel, so avoiding preliminary separations which were liable to introduce errors.

EXPERIMENTAL

EFFECT OF GUM ACACIA ON NICKEL DIETHYLDITHIOCARBAMATE—

In the first instance the effect of larger gum additions on the behaviour of nickel diethyldithiocarbamate was examined by conducting a series of tests with increasing quantities of gum acacia and various percentages of nickel, while otherwise maintaining the conditions of the usual diethyldithiocarbamate procedure. Percentage nickel values were based on the use of a 0·5 g. sample and the subsequent treatment of a 10 ml./100 ml. aliquot portion, prior to dilution to 100 ml. Drum readings were obtained using a 2-cm. cell and spectrum No. 602 blue filters in conjunction with heat-resisting filters H.503.

Since interference was caused by flocculation of colloidal nickel diethyldithiocarbamate, care was taken throughout these tests to obtain drum readings as soon as possible after formation of the colour solution, so avoiding any possible errors due to variable delay. Results of these tests are shown in Fig. 1.

These results clearly demonstrated the serious nature of nickel interference on determinations of copper. They also indicated that, although increased concentrations of gum acacia decreased nickel interference to some degree, the extent to which this occurred provided no material advantage, particularly when relatively high concentrations of nickel were present. It was also apparent that correction curves to compensate for nickel interference would not serve to provide reproducible results.

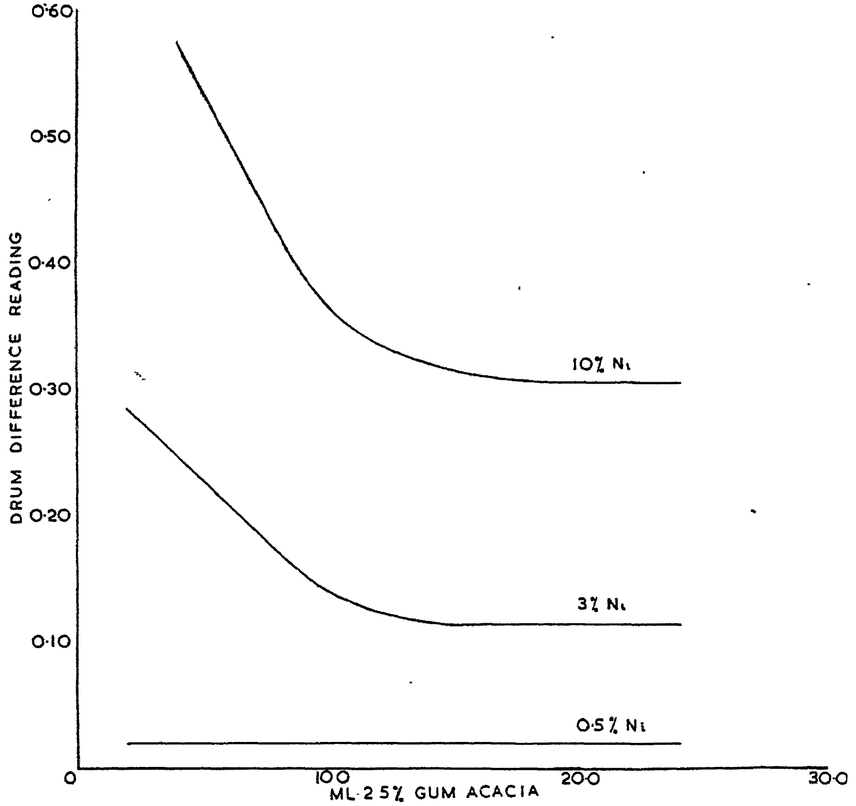

Fig. 1. Effect of gum acacia on nickel diethyldithiocarbamate.

EFFECT OF AMMONIA ON NICKEL DIETHYLDITHIOCARBAMATE—

Some indication had previously been obtained of the soluble nature of the nickel diethyldithiocarbamate complex in ammoniacal solution. A series of tests was conducted, therefore, in which the conditions were similar to those of the earlier tests except that 10 ml. of 2·5 per cent. gum acacia solution were added throughout, and volumes of aqueous ammonia (sp.gr. 0·880) ranging from 2·0 to 30·0 ml. were added; each test solution was finally made up to 100 ml. as before.

In order to ascertain if the time interval between formation and measurement of the colour solution had any significant influence on the accuracy of results, absorption measurements of the test solutions containing 10 per cent. of nickel were obtained again after a period of 3 hours. Results of these tests are shown in Fig. 2.

The presence of excess of ammonia was shown to be ideal for suppression of nickel interference, the quantity necessary being dependent upon the quantity of nickel present.

It was also shown that, provided sufficient ammonia was present, the time interval between formation and measurement of the colour solution was not critical. If, however, insufficient ammonia was present, very serious errors could be introduced.

To obtain more specific information concerning the permissible variation in nickel concentration for any particular concentration of ammonia, five series of tests were conducted, the content of ammonia being maintained constant within each series, while the nickel content varied. The concentrations of aqueous ammonia chosen were 5 ml., 15 ml., 20 ml., 25 ml. and 30 ml. respectively of aqueous ammonia (sp.gr. 0·880) per 100 ml. of solution. Results of these tests are shown in Fig. 3.

These graphs showed that conditions existed whereby nickel interference could be suppressed almost completely and certainly sufficiently for most practical purposes. When dealing with alloys having nickel contents of the order of 10·0 per cent., it was apparent that the use of 30 ml. of aqueous ammonia (sp.gr. 0·880) in the analytical procedure was necessary. In view of this high concentration and the accompanying pungent vapours, it was felt most desirable to have available also a procedure which, by employing a lower concentration of ammonia, would provide an atmosphere more comfortable to the analyst. Results indicated that, to meet these requirements, a concentration of 20·0 ml. of aqueous ammonia (sp.gr. 0·880) per 100 ml. of final solution would be adequate for samples containing up to about 5 per cent. of nickel.

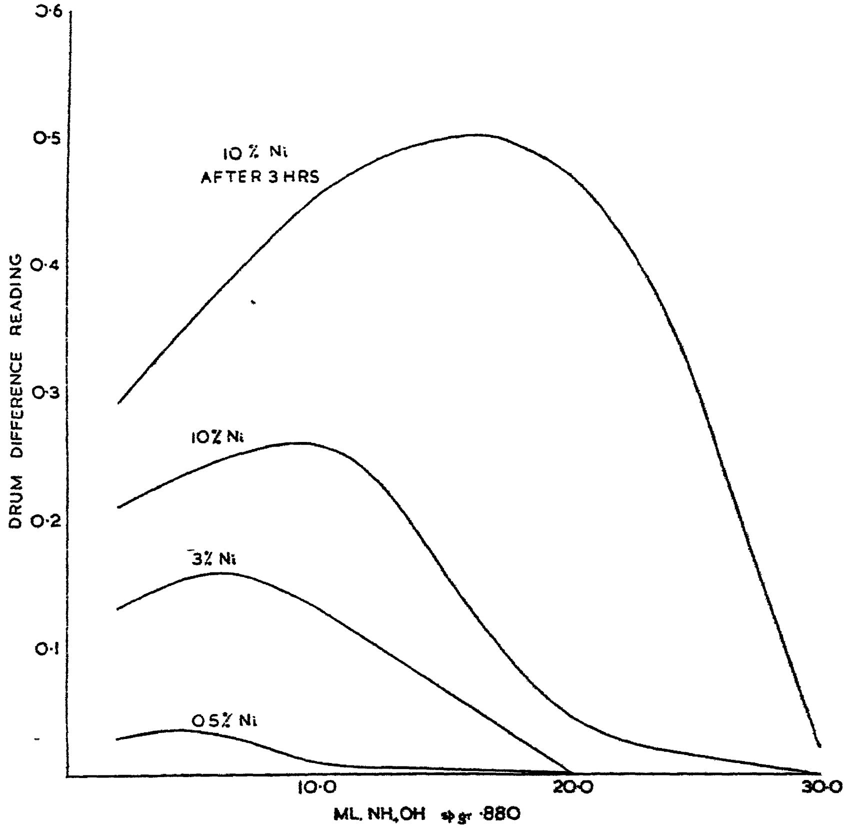

Fig. 2. Effect of ammonia on nickel diethyldithiocarbamate.

Since it was apparent from information obtained that the minimum concentration of ammonia required would be in excess of that previously used in the original diethyldithiocarbamate procedure, analyses were conducted, prior to the preparation of calibration curves, to ascertain whether the concentration of ammonia present in the modified procedure might be such as to promote crystallisation of ammonium salts, when applied to the analysis of ferrous materials. Trouble in this respect was indeed encountered, and, consequently, it was found necessary to reduce the quantity of acid present in the final aliquot. This was accomplished by increasing the weight of sample, and decreasing the aliquot taken for colour measurement, whilst maintaining the volume of solvent acid previously used.

As a basis for further tests, three calibration curves were prepared, by using (*a*) 30 ml., (*b*) 20 ml. and (*c*) 15 ml. of aqueous ammonia (sp.gr. 0·880). The procedure adopted involved the use of solutions of spectrographically pure iron, to each of which was added different volumes of a standard copper solution. Using these curves, determinations of copper were made on copper-bearing solutions of spectrographically pure iron to which were added specific volumes of a standard nickel solution. The concentrations were so chosen that each test solution contained an equivalent of 0·5 per cent. of copper, whilst, over the series, the nickel content varied from 0·0 to 10·0 per cent. Percentage figures were based on the use of a 1-g. sample and the subsequent treatment of a 10 ml./200 ml. aliquot portion prior to dilution to 100 ml. Results of these tests are shown in Table I.

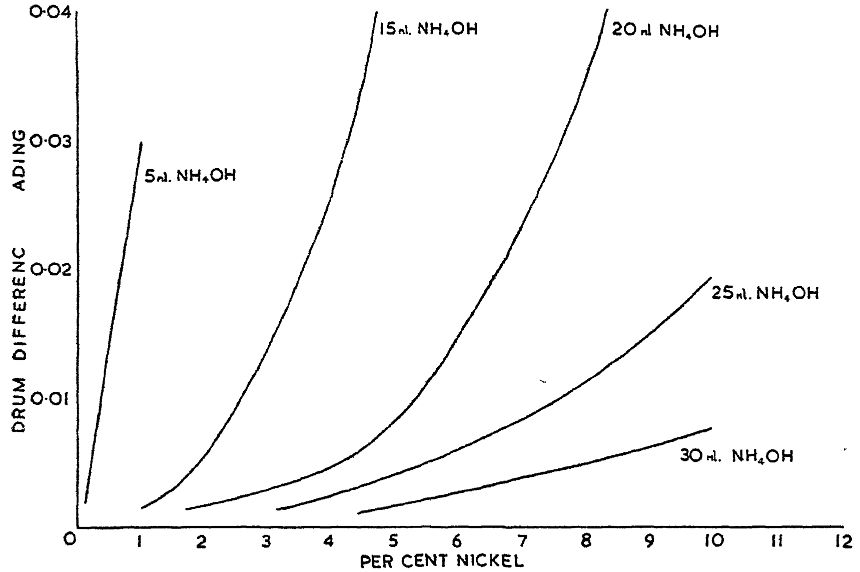

Fig. 3. Nickel interference relative to the concentration of ammonia.

TABLE I

EFFECT OF HIGH CONCENTRATIONS OF AMMONIA ON THE SUPPRESSION OF NICKEL INTERFERENCE IN COPPER DETERMINATIONS

Ni added %	Cu added %	Cu found, % (15 ml. conc. aq. NH_3)	Cu found, % (20 ml. conc. aq. NH_3)	Cu found, % (30 ml. conc. aq. NH_3)
0·0	0·500	0·500	0·500	0·500
0·5	0·500	0·500	0·500	0·500
1·0	0·500	0·505	0·500	0·500
2·0	0·500	0·500	0·505	0·500
4·0	0·500	0·515	0·505	0·505
6·0	0·500	0·560	0·540	0·505
8·0	0·500	0·565	0·530	0·505
10·0	0·500	0·555	0·635	0·505

The above figures, recorded to the nearest 0·005 per cent., showed clearly that, for the determination of copper in presence of nickel, using a 1-g. sample, interference due to nickel could be eliminated, for practical purposes, by the use of 15 ml., 20 ml. and 30 ml. of aqueous ammonia (sp.gr. 0·880) for nickel contents of approximately 2·0, 4·0 and 10·0 per cent. respectively.

STABILITY OF COPPER DIETHYLDITHIOCARBAMATE IN PRESENCE OF HIGH CONCENTRATIONS OF AMMONIA—

Using synthetic solutions prepared as described above, but containing 0·75 per cent. of copper, tests on the stability of the final colour produced under the new conditions were conducted in the presence and absence of nickel. The higher concentration of copper was deemed advisable for this particular series of tests in order that any tendency towards fading of the colour solution might be intensified, and therefore more readily noted. The concentrations of nickel chosen were in each case a maximum for the concentration of ammonia employed. Results of these tests are shown in Table II.

TABLE II

EFFECT OF HIGH CONCENTRATIONS OF AMMONIA ON THE STABILITY OF THE COPPER DIETHYLDITHIOCARBAMATE COLOUR IN PRESENCE AND ABSENCE OF NICKEL

Time interval between development of colour and absorption measurement	15 ml. conc. aq. NH_3 per 100 ml. soln.		20 ml. conc. aq. NH_3 per 100 ml. soln.		30 ml. conc. aq. NH_3 per 100 ml. soln.	
	Cu: 0·75% Ni: nil Cu found %	Cu: 0·75% Ni: 2·00% Cu found %	Cu: 0·75% Ni: nil Cu found %	Cu: 0·75% Ni: 4·00% Cu found %	Cu: 0·75% Ni: nil Cu found %	Cu: 0·75% Ni: 10·00% Cu found %
2 minutes	0·750	0·750	0·745	0·745	0·750	0·750
5 „	0·750	0·750	0·750	0·750	0·750	0·745
10 „	0·750	0·750	0·750	0·745	0·745	0·745
15 „	0·755	0·755	0·750	0·755	0·755	0·750
20 „	0·750	0·755	0·755	0·750	0·755	0·750
25 „	0·755	0·760	0·755	0·755	0·755	0·750
30 „	0·755	0·760	0·755	0·755	0·760	0·755
35 „	0·750	0·765	0·755	0·755	0·760	0·755
40 „	0·750	0·765	0·755	0·755	0·760	0·755
50 „	0·760	0·775	0·765	0·760	0·765	0·765
1 hour	0·760	0·780	0·765	0·760	0·765	0·765
2 hours	0·770	0·920	0·780	0·815	0·780	0·775
3 „	0·775	1·150	0·790	1·080	0·790	0·790

The results indicated that, within reasonable time limits, and in absence of nickel, no serious effect was produced on the colour of copper diethyldithiocarbamate solution by the presence of high concentrations of ammonia.

When dealing with nickel-bearing steels it was apparent that in order to obtain acceptable accuracy the time interval between the formation and measurement of the colour solution should not exceed 30 minutes. In absence of nickel, however, the colour solution remained essentially stable for a period of about 1 hour. It was, of course, clear that to obtain maximum accuracy, measurements should be made as soon as possible after the formation of the colour solution.

It was borne in mind that previous tests had been based on the use of a final sample of 0·05 g. per 100 ml. and that under these conditions a range of copper content of 0·0 to 1·0 per cent. was obtained when using a 2-cm. cell. It appeared reasonable to assume that, by using smaller cells, proportionately higher concentrations of copper might be determined. Experimental tests with concentrations of copper in the range of 1·0 to 2·0 per cent. failed to support this assumption, however, and, indeed, showed it to be invalid owing to the occurrence of turbid solutions caused by breakdown of colloidal copper diethyldithiocarbamate. When dealing with copper contents greater than 1·0 per cent., therefore, the necessity arose to decrease the weight of sample taken rather than to employ the usual weight of sample with smaller cells.

THE METHOD

The method involves solution of the sample in sulphuric - phosphoric acid mixture, oxidation with nitric acid and coloration of an aliquot portion with sodium diethyldithiocarbamate in ammoniacal solution in presence of gum acacia. Metals insoluble in aqueous ammonia are held in solution by means of ammonium citrate, and nickel interference is suppressed by the presence of ammonia.

REAGENTS—

Phosphoric - sulphuric acid—Add 150 ml. of concentrated sulphuric acid and 150 ml. of syrupy phosphoric acid (sp.gr. 1·75) to distilled water and dilute to 1 litre.

Nitric acid—Sp.gr. 1·20.

Ammonium citrate solution—Add 500 ml. of aqueous ammonia (sp.gr. 0·880) to a solution of 500 g. of citric acid in about 200 ml. of distilled water. Dilute to 1 litre with distilled water.

Aqueous ammonia—Sp.gr. 0·880.

Gum acacia solution—Dissolve 12·5 g. of gum acacia in 300 ml. of hot distilled water. Dilute to 500 ml.

Sodium diethyldithiocarbamate solution—Dissolve 0·5 g. in distilled water. Dilute to 250 ml.

Copper—AnalaR copper sheet.

Standard copper solution—Dissolve 0·5 g. of AnalaR copper sheet in a few ml. of nitric acid, boil to expel nitrous fumes and dilute to 1000 ml. with distilled water. Measure off 50 ml. and dilute to 1000 ml. (1 ml. ≡ 0·05 per cent. of copper on a 0·05-g. sample.)

PROCEDURE—

Take 1·0 g. of the sample for alloys containing up to 1·0 per cent. of copper and proportionately less sample for alloys of higher copper contents. Add 20 ml. of phosphoric - sulphuric acid, heat, and when solution is complete, oxidise with 5 ml. of nitric acid. Boil to expel nitrous fumes, filter if necessary, cool, and dilute to 200 ml. Proceed as follows, according to the nickel content of the sample.

Samples containing up to 4·0 *per cent. of nickel*—

Take two 10-ml. aliquot portions, (*a*) test solution and (*b*) compensating solution, and transfer to 100-ml. graduated flasks.

To (*a*) add 10 ml. of ammonium citrate solution, 20 ml. of aqueous ammonia (sp.gr. 0·880), and 10 ml. of gum acacia solution and dilute to approximately 80 ml. Shake well, add 10 ml. of sodium diethyldithiocarbamate solution and finally dilute to 100 ml. with distilled water. Mix well.

To (*b*) add 10 ml. of ammonium citrate solution, 20 ml. of the aqueous ammonia and 10 ml. of gum acacia solution and dilute to 100 ml. with distilled water. Mix well.

Within a period of 30 minutes measure the absorption of each of these solutions under the following conditions:—

Instrument setting:	Water/water 1·00
Light source:	Tungsten filament lamp
Filters:	Ilford Spectrum Blue, No. 602, and heat-resisting, H.503
Size of cell:	2 cm. (for 0 to 1·0 per cent. copper) 4 cm. (for 0 to 0·5 per cent. copper)

Samples containing from 4·0 *to* 10·0 *per cent. of nickel*—

Proceed as above, but add 30 ml. of the aqueous ammonia in place of 20 ml.

Convert difference readings obtained by either of the above procedures to percentage content by reference to calibration curves prepared as described below.

CALIBRATION—

Treat 1·0 g. of spectrographically pure iron as described in the procedure, measure off a number of 10-ml. aliquot portions, and to these, before addition of ammonium citrate, add volumes of the standard copper solution, varying from 0 to 20 ml. Continue the treatment as described for the test solution and obtain the absorption readings. In order to correct for any copper present in the iron and reagents employed, conduct in the same manner a blank determination on an aliquot portion of solution to which no copper has been added. Plot the differences between the absorptions of each test solution and the absorption of the blank solution against the various percentages of copper added. Prepare two calibration curves employing 20 ml. and 30 ml., respectively, of aqueous ammonia (sp.gr. 0·880).

NOTES ON THE METHOD—

(i) Since the nature of the colour solution causes staining of the glass cells, these should be cleaned frequently with dilute nitric acid.

(ii) Since the solutions prepared for absorption measurements contain high concentrations of gum acacia, solid matter dries readily on the outside surfaces of the glass cells. These should, therefore, be carefully examined prior to absorption readings being made.

(iii) Blank determinations should be conducted with each batch of analyses in order to correct for copper present in reagents.

RESULTS

The above method was applied to determinations of copper in various types of alloy steels and cast irons. Whenever possible standard alloys were used, but in view of the limited number of suitable standard materials available, resort was made also to alloy cast irons normally received for analysis. Since certified analyses of these materials were not available, determinations of copper were made by referee chemical methods, the mean of three determinations being accepted for each sample as a basis for comparison with results obtained by the photometric procedure. Results are given in Table III.

TABLE III

Sample	Weight of sample taken g.	Copper present (based on 1·0-g. sample) %	Nickel present (based on 1·0-g. sample) %	Nickel added %	Total nickel present %	Copper found %	
						Using 20 ml. aq. NH_3	Using 30 ml. aq. NH_3
B.C.S. Steel, No. 214	1·00	0·24	0·16	nil	0·16	0·26; 0·26; 0·27	—
B.C.S. Steel, No. 219	1·00	0·15	2·64	nil	2·64	0·18; 0·17; 0·18	—
Amer. Bur. of Stand. Steel, No. 19c ..	1·00	0·16	0·18	nil	0·18	0·17; 0·16; 0·17	—
Amer. Bur. of Stand. 18/8 Steel, No. 101	1·00	0·055	8·44	nil	8·44	—	0·06; 0·06; 0·05
B.C.S. Alloy	0·10	0·47	1·35	nil	1·35	0·49; 0·48; 0·47	—
Cast iron	0·15	0·71	2·01	nil	2·01	0·71; 0·72; 0·72	—
No. 173	0·20	0·95	2·69	nil	2·69	0·96; 0·93; 0·95	—
Cu–Cr–Mo cast iron ..	1·00	0·55	trace	2·00	2·00	0·54; 0·54; 0·55	—
				10·00	10·00	—	0·53; 0·53; 0·54
High duty cast iron ..	1·00	0·82	trace	nil	nil	—	0·84; 0·80; 0·81
				2·00	2·00	0·82; 0·80; 0·83	—
				10·00	10·00	—	0·82; 0·83; 0·81

CONCLUSIONS

1. The method described is applicable to the determination of copper in plain carbon steels, plain cast irons, low alloy steels and irons, and high alloy steels of the 18/8 type.
2. The procedure obviates the necessity of preliminary separation of nickel since interference due to this element can be suppressed for practical purposes by the presence of excess of ammonia, the quantity of ammonia employed being dependent upon the concentration of nickel present in solution.
3. The method provides a colour solution that is stable for a period of 30 to 40 minutes, and suitable for photometric measurement.

SUMMARY

A photometric method for the determination of copper in nickel-bearing steels and cast irons, by means of sodium diethyldithiocarbamate, is described. The procedure makes possible a direct determination of copper without any preliminary separation of either nickel or iron, use being made of the soluble nature of the nickel diethyldithiocarbamate in ammoniacal solution to suppress nickel interference. The effects of additions of gum acacia and aqueous ammonia on the nickel diethyldithiocarbamate and the effect of high concentrations of ammonia on the stability of the copper diethyldithiocarbamate colour solution have been investigated. The procedure provides figures of high accuracy and may readily be employed for batch analysis.

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The Reaction of Nitrous Acid with Alcoholic Alpha-Naphthylamine Hydrochloride and Its Application to the Absorptiometric Determination of Nitrites

BY C. A. PARKER

(1) INTRODUCTION

THE production of azo dyes by the reaction of mixtures of suitable amines with traces of nitrous acid forms the basis of a number of colorimetric methods for the estimation of nitrites.[1,2,3,4,5,6,7] Of these, one of the most widely used is the well-known reaction employing sulphanilic acid and α-naphthylamine.[8,9,10,11,12,13] The colour reaction of nitrous acid with an excess of α-naphthylamine in alcoholic hydrochloric acid, which has already been briefly investigated by the author,[14] suggested a ready means of estimating traces of nitrite, which might have certain advantages over the already existing methods. In the previous work the reaction was carried out in 95 per cent. ethyl alcohol, but for the purpose of nitrite estimation an aqueous medium is often more convenient. Preliminary experiments showed, however, that dilution of the alcohol with water produced a profound effect upon the rate of colour development and that virtually no colour was obtained in an entirely aqueous medium. The purpose of the present work was to investigate in detail the effect of variations in the concentrations of alcohol and hydrochloric acid upon the reaction velocity and maximum colour intensity and thus to develop a sensitive and accurate absorptiometric method for the estimation of nitrite.

(2) RECOMMENDED PROCEDURE FOR THE DETERMINATION OF NITRITES

(*a*) REAGENTS—

α-*Naphthylamine*—"A.R." quality material recrystallised from boiling light petroleum (b.p. 40° to 60° C.) to give colourless needles.

Hydrochloric acid—"A.R." quality, 10 *N*; the normality must not deviate from this value by more than ±10 per cent.; if a greater deviation is found an appropriate adjustment should be made in the volume taken to prepare the mixed reagent.

95 *per cent. Alcohol*—Redistilled from sodium hydroxide.

Mixed reagent—Dissolve 1·25 g. of α-naphthylamine in 1000 ml. of alcohol. To this solution add 8·8 ml. of hydrochloric acid. The reagent is stable in the absence of air and light.

(*b*) PREPARATION OF SAMPLE—

If the sample is strongly acid or alkaline it should first be neutralised to methyl orange by titration with sodium hydroxide or hydrochloric acid. For this titration dilute methyl orange solution may be added at the rate of 0·5 ml. of 0·002 per cent. solution per 20 ml. of sample. This will produce a small drum reading on the "Spekker," which will be automatically compensated when the blank is measured.

When very small quantities of nitrite are to be estimated it is desirable to titrate a 20-ml. aliquot of the sample to methyl orange and then to carry out the nitrite determination on a separate 20-ml. aliquot to which has been added the appropriate amount of acid or alkali to give a total volume not exceeding 22 ml.

(*c*) PROCEDURE—

Pipette a 20-ml. aliquot of the neutral aqueous sample into a 50-ml. calibrated flask. Pipette in 20 ml. of the mixed alcoholic reagent. Mix, allow to stand in the dark for 10 minutes at room temperature and then place in a water-bath at 60° ± 2° C. for 30 minutes. Cool to 20° C. and make up to the mark with distilled water. Measure the drum reading with the "Spekker," using Ilford Yellow-Green filters No. 605 in conjunction with either the tungsten or mercury lamp, and the appropriate cell thickness to give a drum reading of less than 1·0. Carry out a blank determination using 20 ml. of nitrite-free water containing (if appropriate) 0·5 ml. of 0·002 per cent. methyl orange solution. Multiply the drum difference reading by the factor obtained from a calibration graph prepared by using standard solutions of sodium nitrite, and thus obtain the concentration of nitrite in the sample.

(3) ADVANTAGES OF THE PROPOSED METHOD

The coloured solutions are stable for several days and obey Beer's law exactly. Reproducible straight-line calibration graphs can be obtained with all sizes of Spekker cell and the slopes are directly proportional to cell thickness. Relatively high or very low concentrations of nitrite may thus be estimated with equal accuracy and without precipitation of the dye. After selection of a suitable cell thickness, drum readings may be converted directly to nitrite concentration by means of the appropriate factor without reference to the calibration curves. In contrast to this, the α-naphthylamine-sulphanilic acid reaction, when carried out in an aqueous medium, gives precipitation of the dye except with very low nitrite concentrations.[13] It was in an attempt to overcome this difficulty that Richardson and Hollings[10] recommended the use of methylated spirit.

The sensitivity of the reaction is such that, with a 4-cm. cell, less than 1 part of nitrite in 200 million may be detected. The method has the further advantage that the absorption takes place at the wavelength of green light and interference by faint yellows or browns in the solution is minimised. Such interference is generally negligible, but if the sample is strongly coloured accurate compensation may be made by deducting the absorption shown by a solution derived from a similar aliquot of the sample to which has been added alcoholic hydrochloric acid but no α-naphthylamine.

The absorption band of 4-amino-1 : 1′-azonaphthalene hydrochloride (peak at 5500 A.) is ideally situated for measurements with the 5461 A. mercury line. This line may be readily isolated by means of the Ilford Yellow-Green filter No. 605. Calibration graphs obtained in this way correspond almost exactly to peak extinction and have slightly greater slopes than those obtained with the tungsten lamp.

(4) EFFECT OF VARIOUS FACTORS UPON THE RATE OF COLOUR DEVELOPMENT

Measurements of reaction velocity were made in the first place at 20° C., with the Spekker photo-electric absorptiometer, using "tall-form" 1-cm. cells which were maintained at constant temperature by means of a specially constructed bath.[15] The procedure used was as follows.

The requisite quantity of a standard alcoholic solution of sodium nitrite (containing from 0·15 to 0·5 mg. of $NaNO_2$ in 90·0 per cent. w/w alcohol) was placed in a 100-ml. calibrated flask together with the appropriate quantity of water and the volume made up to 79 ml. with 90·0 per cent. w/w alcohol. The flask and contents were brought to the required temperature in a thermostat. A mixed reagent of α-naphthylamine (50 mg. per 20 ml.) and hydrochloric acid (of appropriate concentration) in 90·0 per cent. w/w alcohol was also adjusted in temperature. Twenty ml. of the mixed reagent were then pipetted rapidly into the nitrite solution. The volume was made up to 100 ml. with 90·0 per cent. w/w alcohol and after complete mixing a quantity was poured into one of the dry Spekker cells in the constant-temperature bath. Drum readings (water to water = 1·00) were taken at short intervals of time until the colour was fully developed.

The reaction velocity constant was calculated as follows. First, the drum readings were plotted against time and a mean reaction - time curve drawn (in this way the random errors of the single Spekker measurements were eliminated). Then, assuming that at any stage of the reaction the colour intensity (*i.e.*, $1 - D_n$) is proportional to the quantity of nitrite that has reacted, the following equation for a first-order reaction applies:

$$\log_{10}(D_n - D_n') = \frac{-kt_n}{2\cdot303} + \log_{10}[(1 - D\infty)(1 - e^{-kT})]$$

where D_n = drum reading obtained at time t_n.
D_n' = drum reading obtained at time t_n'.
$D\infty$ = drum reading corresponding to infinite time.
k = first order reaction velocity constant.
T = an arbitrary and constant time interval.

From the graph of D_n against t_n, values of $\log_{10}(D_n - D_n')$ were calculated and plotted against t_n. From the slope of the straight line so obtained the reaction velocity constant was calculated.

α-Naphthylamine was always present in large excess and the reaction was found to be of the first order with respect to the nitrous acid. The reaction velocity constant was first measured in 90·0 per cent. w/w alcohol containing various concentrations of hydrochloric acid. These experiments were then repeated with different concentrations of alcohol. The complete set of results is shown in Fig. 1 (curves 1, 2, 3, 4 and 5). It will be noted that in 90 per cent. w/w alcohol the reaction rate is a maximum at an acid normality of 0·08 (curve 1). It decreases rapidly with decrease of acidity below this point, and more slowly with increase of acidity above this point. On decreasing the concentration of alcohol all the reaction rates are decreased, and at the same time the normality corresponding to maximum velocity moves to lower values, until finally at an alcohol content of 24 per cent. w/w no maximum appears above a normality of 0·02. The theoretical significance of all these effects is discussed in the following section.

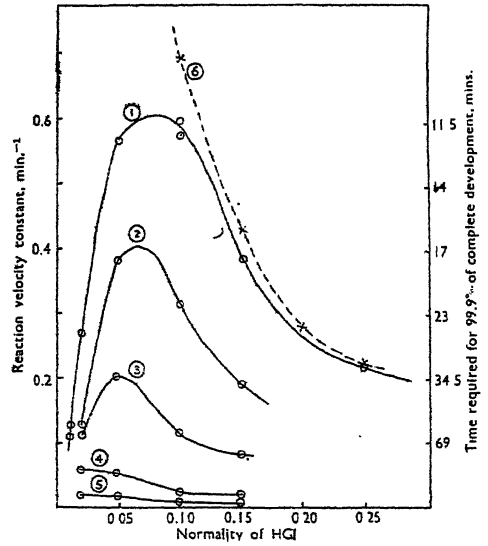

Fig. 1. Effect of concentration of hydrochloric acid and alcohol upon the rate of reaction of nitrous acid with α-naphthylamine at 20° C.
Curve (1) in 90·0% w/w ethyl alcohol.
„ (2) in 61·1% „ „ „
„ (3) in 50·3% „ „ „
„ (4) in 32·6% „ „ „
„ (5) in 23·9% „ „ „
„ (6) Rate of coupling of α-naphthylamine diazonium chloride with a large excess of α-naphthylamine in 90% w/w ethyl alcohol.

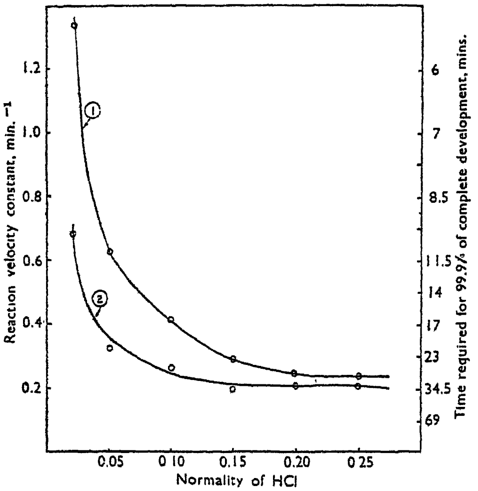

Fig. 2. Effect of concentration of hydrochloric acid and alcohol upon the rate of reaction of nitrous acid with α-naphthylamine at 60° C.
Curve (1) in 32·6% w/w ethyl alcohol.
„ (2) in 23·9% „ „ „

From the point of view of an analytical method for the estimation of nitrous acid, a compromise has to be made between high rate of development on the one hand and low alcohol concentration on the other. With high water content at 20° C. the reaction is too slow for convenience. However, 60° C. appears to be a convenient temperature for the test, and reaction velocity experiments at this temperature showed that the colour is fully developed within 30 minutes, even in solutions containing as little as 24 per cent. of alcohol and at fairly high acidities (see Fig. 2). The further investigations, described in sections (6) to (9) below, were therefore confined to 60° C.

(5) DISCUSSION OF THE MECHANISM OF THE REACTION

It has been shown[14] that the colour is due to the formation of the blue-violet hydrochloride of 4-amino-1 : 1′-azonaphthalene in the following way:—

(i) $C_{10}H_7.NH_2 + HNO_2 + H^+ \longrightarrow C_{10}H_7.N_2^+ + 2H_2O$.

(ii) $C_{10}H_7.N_2^+ + C_{10}H_7.NH_2 \longrightarrow C_{10}H_7.N_2.C_{10}H_6.NH_2 + H^+$.

The results described in section (4) above may be explained on the basis of this two-stage reaction. At high acidities the initial diazotisation of the α-naphthylamine is very rapid and the subsequent coupling of the diazonium chloride with a further molecule of α-naphthylamine is the rate-determining step, the rate of coupling being retarded by increase of acidity. On the other hand, at very low acidities, the coupling reaction is very rapid, the diazotisation being retarded and becoming the rate-determining step. This theory has been confirmed by measuring the rate of coupling at varying acidities in the following way—

> 6·2 mg. (1·2 mol.) of α-naphthylamine in 5 ml. of 0·2 *N* hydrochloric acid were treated with 5 ml. of an aqueous solution of 2·5 mg. (1 mol.) of sodium nitrite. After 10 minutes standing at room temperature the diazotisation was complete. 1 ml. of the resulting naphthalene diazonium chloride solution was added to a 100-ml. calibrated flask containing 50 mg. of α-naphthylamine in solution in 91 per cent. w/w alcohol containing the appropriate concentration of hydrochloric acid, the latter solution having previously been brought to 20° C. in a thermostat. The mixture was rapidly made up to the mark with 91 per cent. w/w alcohol, mixed, and poured into a dry Spekker cell in the constant-temperature bath. Drum readings were taken at frequent intervals and the reaction velocity constant calculated by the method already described.

Below a normality of 0·10 in 90 per cent. alcohol the rate of coupling increases very rapidly (see Fig. 1, curve 6), but at normalities greater than 0·20 the rate of coupling approximates to the over-all reaction rate.

The reduction of the over-all reaction velocity with decrease of alcohol content, and the accompanying movement of the peak in the curves towards lower acidities (see Fig. 1) suggests an increase in the ionisation of the hydrochloric acid at the lower alcohol concentrations. This would, of course, have the effect of lowering the rate of coupling until finally this reaction would become the rate-determining step throughout the range of acidity investigated.

The rates of coupling of diazonium salts with tertiary amines have been investigated by Goldschmidt[16] who noted a retardation of the reaction on increasing the acidity. Similar effects have been observed by other workers in the reaction of nitrous acid with a mixture of sulphanilic acid and α-naphthylamine. Thus, Moffit[11] recommends diazotisation of sulphanilic acid in dilute sulphuric acid solution followed by buffering with sodium acetate to allow the coupling reaction with α-naphthylamine to take place.

Rider and Mellon[13] have shown that the order of addition of the reagents in this reaction is of great importance: if the α-naphthylamine is added before completion of the diazotisation of the sulphanilic acid, low colour intensities are obtained owing to partial diazotisation of the α-naphthylamine. They recommend preliminary reaction of the sulphanilic acid in acid solution followed by the addition of α-naphthylamine and subsequent buffering with sodium acetate to facilitate the coupling reaction. The application of a similar principle was attempted in the present investigation, acid alcoholic α-naphthylamine solution being added to the nitrite solution followed by sodium acetate to allow coupling to give the brown azo-base. While some success was attained with this procedure, it was felt that the method now recommended had the advantage of simplicity and reproducibility.

(6) EFFECT OF CONCENTRATION OF HYDROCHLORIC ACID UPON THE INTENSITY OF THE FULLY DEVELOPED COLOUR

4-Amino-1 : 1′-azonaphthalene is a weak base and has indicator properties, being red-brown in neutral or alkaline solution and blue-violet in acid solution. The absorption spectrum of the free base exhibits a maximum at 4650 A. in neutral or alkaline alcoholic solution, whilst in strongly acid solution a peak appears at 5500 A. owing to the cation of the basic salt (see Fig. 3). However, the basicity of the compound is so small that appreciable hydrolysis of the hydrochloride is apparent even in 0·1 *N* alcoholic hydrochloric acid. Consequently the intensity of the peak at 5500 A. and the Spekker reading obtained with the yellow-green filters (Fig. 4, curve 2) varies with the acid concentration.

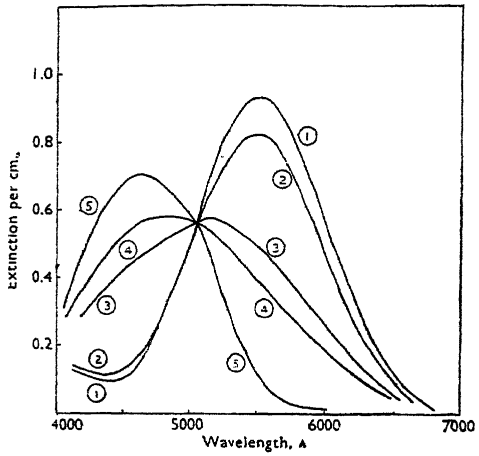

Fig. 3. Effect of acidity upon the absorption spectrum of 4-amino-1 : 1′-azonaphthalene in 90% alcohol.

Curve (1) *N* HCl
" (2) 0·2 *N* HCl
" (3) 0·01 *N* HCl
" (4) 0·005 *N* HCl
" (5) 0·1 *N* NH_4OH

All solutions contain 0·00093% of dye.

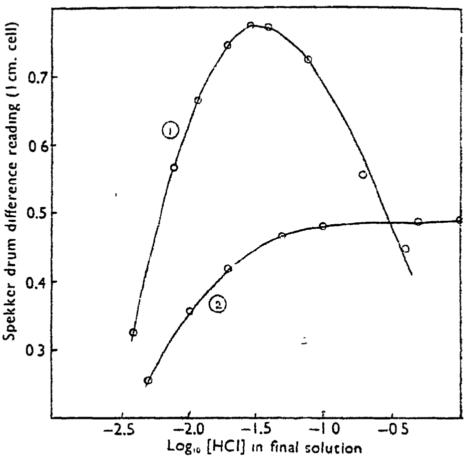

Fig. 4. Effect of concentration of hydrochloric acid upon colour intensity developed from sodium nitrite.

Curve (1) 0·15 mg. of sodium nitrite treated by prescribed method with mixed reagents containing the appropriate concentration of HCl.
" (2) 0·0005% solutions of pure 4-amino-1 : 1′-azonaphthalene in 33% w/w alcohol containing the stated concentrations of HCl.

Solutions obtained by the treatment at 60° C. of sodium nitrite with α-naphthylamine in varying acid concentrations (in a mixture of equal volumes of 95 per cent. alcohol and water—see "Procedure for Determination of Nitrites") show a further effect (Fig. 4, curve 1). At low acidities the extinction, as measured on the Spekker, falls owing to the shift in the absorption band of the dye, but at high acidities the extinction also decreases. A similar effect is shown when the reaction is carried out at 20° C. in those cases where the velocity of the coupling reaction is small (*i.e.*, at low alcohol concentrations). It may be explained by assuming the naphthalene diazonium chloride to undergo two simultaneous reactions, namely, the coupling reaction to give the dye and a decomposition to give colourless products such as α-naphthol.

$$\text{amino-azonaphthalene} \xleftarrow{\text{A}} \begin{matrix}\text{diazonium}\\ \text{chloride}\end{matrix} \xrightarrow{\text{B}} \begin{matrix}\alpha\text{-naphthol,}\\ \text{etc.}\end{matrix}$$

If reaction A is slow, the effect of B becomes appreciable and low colour intensities are obtained.

The optimum point corresponds to a final normality of 0·03 to 0·04. A normality of 0·035 has been provided for in the recommended method.

(7) EFFECT OF CONCENTRATION OF ALCOHOL UPON THE INTENSITY OF THE FULLY DEVELOPED COLOUR

Fixed amounts of nitrite were treated by the recommended procedure except that different varying amounts of alcohol were used. The results are shown in the following table.

Percentage by volume of "95 per cent. alcohol" in the reaction mixture	"Spekker" drum divisions
30	0·60
35	0·68
40	0·72
45	0·745
50	0·75
60	0·745
70	0·755

For solutions containing less than 50 per cent. by volume of 95 per cent. alcohol the concentration becomes critical and low colour intensities are obtained. A mixture of equal volumes of 95 per cent. alcohol and water was therefore chosen as the reaction medium in the recommended method. After final dilution to 50 ml. with water this corresponds to from 33 to 34 per cent. w/w of alcohol.

(8) EFFECT OF TIME OF STANDING AT ROOM TEMPERATURE BEFORE HEATING TO 60° C.

It was thought possible that reaction at 60° C. might cause abnormally low colour development owing to acceleration of the decomposition of the naphthalene diazonium chloride to colourless products. Since, with the chosen concentrations of reagents, the transformation of nitrous acid to dye is more than 50 per cent. complete within 10 minutes at 20° C., it was decided to allow the solutions to stand for this period at room temperature before completing the reaction at 60° C. The decomposition of the diazonium chloride was thus minimised and a source of variation in the results was removed. Prolonged standing at room temperature before development at 60° C. produced only a slight increase in colour intensity. After heating at 60° C. the solutions were found to be stable for several days when stored in the dark at room temperature; but a gradual fading occurred later.

(9) INTERFERING IONS

The effect of the presence of a number of common ions was investigated, using nitrite concentrations of 0·3 p.p.m. (with 4-cm. cell) and 2·0 p.p.m. (with 1-cm. cell). The salts investigated have been grouped below according to the concentrations that produce an error of less than 5 per cent. in the determination.

10,000 *p.p.m.*	$NaCl$, $NaNO_3$, Na_2CO_3, $NaBr$, $NaC_2H_3O_2$. $MgCl_2$, $CaCl_2$, $BaCl_2$, NH_4Cl.
1000 *p.p.m.*	Na_2SO_4, $Na_2B_4O_7$, NaH_2PO_4, Na_2SiO_3. $MnSO_4$, $Co(NO_3)_2$, $NiSO_4$, $Cr_2(SO_4)_3$, $ZnSO_4$, $Pb(NO_3)_2$.
100 *p.p.m.*	NaF, Na_2SO_3.
10 *p.p.m.*	$FeCl_2$, $FeCl_3$, $CuSO_4$.
$<$10 *p.p.m.*	Na_2S.

Alkalis and alkaline earth metals and nitrate ion are without effect even in concentrations as high as 1 per cent. High concentrations of chloride cause a slight increase in the colour intensity; a similar effect has been noted by Morpeth[12] with the α-naphthylamine - sulphanilic acid reaction. Sulphates in very high concentration cause a considerable diminution in the Spekker reading owing to the suppression of the formation of the hydrochloride of the dye (the solutions having a reddish tint).

Large quantities of basic salts will, of course, interfere with the test, either by completely inhibiting the formation of the dye, or by decreasing the formation of its hydrochloride. These effects are minimised in the proposed method by first neutralising the solution to methyl orange, the small absorption due to the latter being deducted from the drum reading obtained. When very low concentrations of nitrite are concerned the titration may be carried out on a separate aliquot and the requisite volume of hydrochloric acid added to the sample before development of the colour. Generally speaking, however, this neutralisation will be unnecessary.

Interference caused by inert coloured ions may, of course, be automatically compensated by carrying out a blank determination omitting only the α-naphthylamine. Certain ions (notably $Fe^{\cdot\cdot\cdot}$) produce a precipitate with the reagent, and their concentrations must therefore be kept within low limits.

When estimations of nitrite are required in presence of high concentrations of ions such as sulphate or phosphate, which change the tint of the dye, accurate results may be obtained by preparing a fresh calibration curve relating to the appropriate quantity of interfering ion present.

SUMMARY

A detailed study has been made of the colorimetric reaction between nitrous acid and an aqueous-alcoholic solution of α-naphthylamine hydrochloride. The effects of acid normality, alcohol concentration and temperature upon the reaction velocity and maximum colour

intensity are described, and some observations have been made concerning the mechanism of the reaction.

As a result of these investigations a sensitive and accurate absorptiometric method for the estimation of nitrite has been developed. It has the advantages of rapidity, colour stability and reproducibility. Few ions interfere seriously and the dye obeys Beer's law over a wide concentration range.

The investigations described above were carried out at the Admiralty Materials Laboratory and permission to publish this paper is gratefully acknowledged. The author wishes also to acknowledge the valuable assistance given by Mr. A. P. Goddard with the experimental work.

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ADMIRALTY MATERIALS LABORATORY
HOLTON HEATH, POOLE, DORSET

March, 1948

The Determination of Poisonous Metals (Copper, Lead and Zinc) in Edible Gelatin

BY A. SCOTT-DODD

THE Edible Gelatin (Control) Order, 1947, No. 161, January 30th, 1947, fixed the following limits for poisonous metals—

Copper	30 parts per million
Lead	10 „ „
Zinc	100 „ „

The chief source of trouble in testing gelatin is the viscous nature of its solutions. Hydrolysis with hydrochloric acid and water removes this difficulty and at the same time precipitates some of the proteid matter. After filtration from this it is found that all the metallic impurities are present in the filtrate. A small amount of iron is usually present, and must either be removed or kept from interfering with the subsequent reactions. It is found that, from an aqueous solution acidified with hydrochloric acid, all the copper and iron are extracted by a solution of dithizone in chloroform. It is a simple matter to separate the iron from the copper by precipitation with ammonia, leaving the copper salts in solution. As the contents of the separator now contain only the zinc and lead, these metals are readily extracted by dithizone after a slight excess of ammonia has been added. It is easy to determine the quantity of each, as there should be neither copper nor iron present to interfere with the reactions of potassium ferrocyanide or sodium sulphide. As a precaution, however, it is advisable to add some potassium cyanide, when testing for lead with sodium sulphide.

The details of the method are as follows.

METHOD

REAGENTS—

Solution of diphenylthiocarbazone (dithizone) in chloroform, 0·1 per cent.
Solution of sodium diethyldithiocarbamate, 0·1 per cent. in water.
Solution of potassium ferrocyanide, 1 per cent.
Solution of potassium cyanide, 10 per cent.
Solution of sodium sulphide, 1 per cent.
Standard solutions of copper, zinc and lead, prepared as below.

Copper standard—Dissolve 0·7856 g. of $CuSO_4.5H_2O$ in distilled water, add a few drops of concentrated sulphuric acid and make up to a litre. Dilute ten-fold with distilled water. This makes a standard solution containing 0·02 g. of Cu per litre.

Zinc standard—Dissolve 0·220 g. of $ZnSO_4.7H_2O$ in distilled water and make up to a litre. This makes a standard solution containing 0·05 g. of Zn per litre.

Lead standard—Dissolve 0·3662 g. of lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$ in distilled water, add a few drops of acetic acid and make up to a litre. Dilute this solution 100-fold with distilled water. This makes a standard solution containing 0·01 g. of Pb per litre.

PROCEDURE—

To 25 g. of gelatin add 10 ml. of concentrated hydrochloric acid and 100 ml. of water, digest on a steam-bath for one day and overnight, in a conical flask. Cool and make up to 250 ml. Filter this 10 per cent. stock solution as required.

Extract 60 ml. of the filtrate (equal to 6 g. of sample) in a separator with about 5 ml. of dithizone reagent, four times.

Collect the extract in a small "CO_2 flask" and distil off the chloroform in a Soxhlet tube. Transfer to a platinum basin, by washing out with small successive quantities of chloroform, and ignite gently with the aid of a few drops of concentrated sulphuric acid and a few drops of nitric acid until all the organic matter and nitric acid disappears. Heat to boiling with water and a few drops of hydrochloric acid, cool and make up to 50 ml. (Solution A.)

Copper—Take 25 ml. of solution A (equal to 3 g. of sample), add a distinct excess of ammonia solution, heat, filter through a small filter paper and wash into a Nessler tube to remove any iron that may be present. Add 2 ml. of 0·1 per cent. sodium diethyldithiocarbamate solution, and compare with the standard copper solution (0·02 g. per litre); 3 ml. of this standard solution ≡ 20 parts of copper per million of the sample.

Zinc and lead—To the contents of the separator add ammonia solution to slight alkalinity. Extract four or five times with 5 ml. of dithizone reagent. Treat the extract as above and make up, as before, to 50 ml. (Solution B.)

Zinc—To 25 ml. of solution B (equal to 3 g. of sample) add a few drops of hydrochloric acid, 1 g. of ammonium chloride and 1 drop of potassium ferrocyanide solution. Compare with the standard zinc solution (0·05 g. per litre); 3 ml. of this standard solution ≡ 50 parts of Zn per million of the sample.

Lead—To 25 ml. of solution B and 1 g. of ammonium acetate, with sufficient ammonia to make the solution distinctly alkaline, add 1 ml. of 10 per cent. potassium cyanide solution and then 1 ml. of 1 per cent. sodium sulphide solution. Compare with the standard lead solution (0·01 g. of Pb per litre); 3 ml. of this standard solution ≡ 10 parts of Pb per million of the sample.

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June, 1947

Notes

THE ESTIMATION OF SELENIUM IN LEAD ALLOYS*

SELENIUM in small amounts is now sometimes added to lead alloys as a grain refiner, and the following method for its determination has been found to give good results. The alloy, preferably in the form of filings or very small pieces, is dissolved in perchloric acid and diluted to a suitable volume with diluted hydrochloric acid (1 + 2). Sulphur dioxide is passed into the solution and causes precipitation of the selenium as a scarlet precipitate. The selenium is filtered off, washed free from lead salts and determined according to the method of B. S. Evans (*Analyst*, 1942, **67**, 88).

Method—

Dissolve 3 g. of the alloy in 30 ml. of perchloric acid (which has previously been concentrated by evaporation to strong fuming) and warm until all action ceases. Heat until all black particles have dissolved. Cool, add 400 ml. of diluted hydrochloric acid (133 ml. of the concentrated acid plus 267 ml. of water) and boil until all salts have dissolved and the solution is quite clear. While the solution is still hot pass in a brisk stream of sulphur dioxide for 3 or 4 minutes to precipitate the selenium, and tellurium if present. Place on the hot-plate for ½ hour, then filter on pulp, keeping the solution hot in order to avoid, as far as possible, the separation of lead chloride. Wash thoroughly with boiling hot diluted hydrochloric acid (1 in 20) until the pulp and precipitate are free from lead salts; 200 ml. or more will be required. From now on, the process is as described by B. S. Evans, *i.e.*, the precipitate is dissolved from the filter by means of bromine solution in dilute hydrochloric acid (5 ml. of a saturated solution of bromine in concentrated hydrochloric acid + 10 ml. of water), the solution being collected in a flask, and the filter is washed with 5 per cent. hydrochloric acid solution so that finally 100 to 150 ml. of filtrate coloured deep orange with bromine are obtained; the filter should be white. Remove excess of bromine from the solution by bubbling through it a rapid stream of air until the colour is pale lemon-yellow. Next add 20 ml. of 4 per cent. potassium iodide solution and shake well. Leave for 1 to 1½ hours with occasional shaking, filter off the scarlet precipitate on a pulp filter and wash (*a*) with 100 ml. of a solution containing 5 per cent. of hydrogen chloride and 5 per cent. of ammonium chloride, (*b*) twice with 5 per cent. ammonium chloride solution to remove excess of acid and (*c*) five or six times with 5 per cent. ammonium nitrate solution to remove chlorides. Suck the washed filter (with selenium precipitate) dry by momentarily attaching the stem of the funnel to a filter pump, blow it out into a beaker, wiping the funnel with a piece of moist filter paper, run 10 ml. of 10 per cent. potassium cyanide solution over the walls of the funnel into the beaker and rinse with hot water. Add sufficient water to enable the paper pulp in the beaker to be disintegrated with a glass rod, stir in 0·4 g. of sodium nitrite and place in a steam-bath. After solution of the precipitate run in 25 ml. of nitric acid (sp.gr. 1·2), stir thoroughly and heat to boiling. Filter into an Erlenmeyer flask of about 600-ml. capacity, wash the pulp thoroughly with hot water and discard it. Evaporate the solution to about 40 ml., cool and roughly measure its volume, place 1 g. of urea in the Erlenmeyer flask, pour the solution back into that flask and rinse the measuring tube with cold water into the flask so as to give a total volume of 100 ml. Add 2 ml. of 10 per cent. potassium cobalticyanide solution in case a trace of copper has found its way into the solution. Shake, leave for about 1 minute, add 10 ml. of 4 per cent. potassium iodide solution and 5 ml. of chloroform and shake thoroughly again. Leave for 5 minutes with repeated shakings and titrate the liberated iodine with 0·01 N sodium thiosulphate, with vigorous shaking near the end, and using starch for the final end-point. The end-point is orange, owing to precipitated selenium, which accumulates in the chloroform.

Factor: 1 ml. of 0·001 N sodium thiosulphate ≡ 0·0001975 g. selenium.

The following results were obtained with samples of lead alloy containing 3 per cent. of tin and 3 per cent. of antimony to which selenium had been added.

Alloy taken	Selenium added	Titration	Selenium found	Selenium	
				added	found
	g.	ml.	g.	% of alloy	% of alloy
3·0	0·0009	4·8	0·00095	0·030	0·031
3·0 †	0·0006	3·1	0·00061	0·020	0·020
3·0	0·0024	12·1	0·00239	0·080	0·080

The method is unsuitable for alloys containing higher percentages of tin, owing to the formation of insoluble metastannic acid during solution in the perchloric acid.

Thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish this note.

F. W. BOX

July, 1948.

A TRANSMITTING MANOMETER FOR MICRO OXYGEN UPTAKE EXPERIMENTS

APPARATUS for the measurement of the oxygen uptake of oils and fats has been extensively studied.[1,2] On attempting to perform the measurement on a micro-scale, the capacity of the indicating manometer is no longer negligible in relation to that of the remainder of the system, and it becomes desirable to enclose the entire system in a constant-temperature oven.[3] Under these conditions an indirect method of reading the manometer is necessary. An electrical method was accordingly developed, the connecting wires entering the oven through the thermometer aperture.

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* Communication from the Armament Research Department (formerly Research Department, Woolwich).

† This alloy contained also 0·02% of tellurium and 0·02% of arsenic.

The construction of the manometer U-tube is shown in Fig. 1. To the platinum contacts A A are attached filaments B B of 36 S.W.G. platinum wire. The ends of the latter are sealed into the glass at the bend of the tube, so that the filaments are under very light tension and are central in the limbs. A third contact, C, sealed through the glass, enables electrical contact to be made with the mercury, which is introduced to a level about half-way up the limbs. One of the side-tubes is connected to the absorption vessel, the other being left open to the atmosphere. (If the manometer is to be used differentially, appropriate connections are made to both side-tubes.)

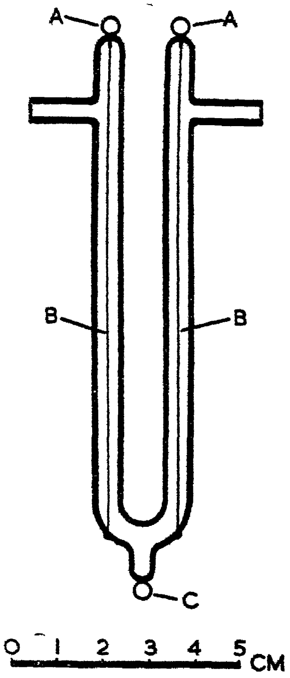

Fig. 1.

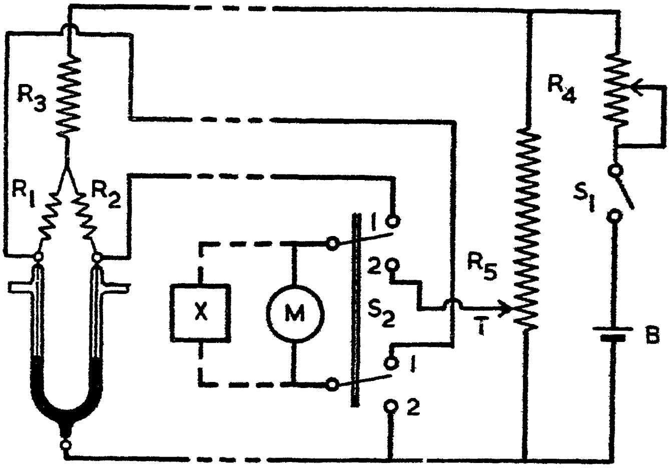

Fig. 2.

R_1 and R_2, equal, about 1·5 ohms.
R_3, 13 ohms.
R_4, 10 ohms.
R_5, 200 ohms.
M, Galvanometer.
S_1, On-off switch.
S_2, Double-pole double-throw switch.
X, Recorder.

Electrical connections are shown in Fig. 2. The filaments, together with resistances R_1 and R_2, constitute a Wheatstone network, the state of balance of which is indicated by meter M. Pressure changes displace the mercury column, thereby altering the relative effective lengths of the filaments in the circuit and consequently altering the state of balance. The meter is calibrated against a series of known pressure differences. Resistance R_3 limits the potential drop across the network to about 0·4 volt, thus avoiding heating effects.

In order to maintain the calibration the voltage applied to the network must be constant. The applied voltage may be adjusted or checked by moving switch S_2 from position 1 to position 2 and adjusting variable resistance R_4 until the meter gives a full-scale or other convenient predetermined reading. The position of tap T near one end of resistance R_5 is adjusted to suit the particular meter in use. Except for R_4, which is a radio component, all resistances are made from 35 S.W.G. Eureka wire. In no case is the value critical.

Results obtained using a Griffin & Tatlock "Microid" central zero galvanometer (1·6 microamperes per scale division) are shown in Fig. 3. Pressures were measured on a water manometer and converted into millimetres of mercury. In the range shown, indications of pressure change were instantaneous and reproducible. Use of a Cambridge "Spot" galvanometer (full-scale deflection, approx. 1 microampere) equipped with a suitable shunt enabled the sensitivity to be increased. With pressure differences less than about 4 mm. of mercury, results were somewhat erratic, presumably owing to slight sticking of the mercury. If it is desired to operate at such low pressures the difficulty can be overcome by mounting the manometer on an upright to which a buzzer operated by two dry cells is also attached. The slight agitation thus produced causes the mercury level, and hence the meter reading, to respond instantly to small pressure changes. A typical calibration curve thus obtained is shown in Fig. 4.

The apparatus is obviously of general application to other cases involving pressure indication, *e.g.*, measurement of gas flow or gas thermometry. Not only can readings be obtained at a distance, but it should be possible also to replace meter M by a recorder, so that results may be studied at leisure.

Subsequent to the completion of this work, our attention was drawn to a paper,[4] describing a manometer device operating a recorder through a Wheatstone network, but using an entirely different principle. A platinum filament, electrically maintained at a slightly elevated temperature, extends down one limb of the manometer U-tube, in which a paraffin filling is used. Movement of the paraffin level alters the immersion, and hence the cooling, of the filament. Change in resistance thus caused alters the state of the bridge balance. This device is very sensitive, but fluctuations in the heating current must be carefully avoided.

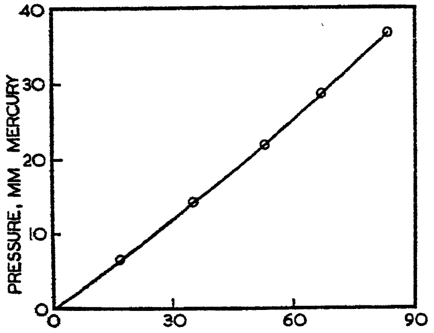

Fig. 3.

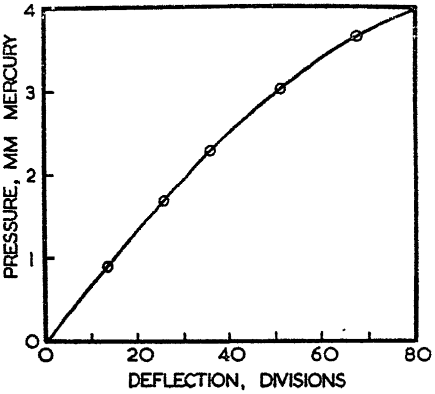

Fig. 4.

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A MICRO-BLOWPIPE

The apparatus described provides a small, stable, hot flame suited to the fabrication of micro-balance beams, micro-dispensing and drop testing devices, and the like, from capillaries and fine glass rod.[1,2,3] The cost is negligible and the air supply may be provided by a device operating from a filter pump.

Details of construction are shown in Fig. 1. The gas chamber consists of a steel washer A (1½ inch outside diameter × $\frac{13}{16}$ inch inside diameter × 16 S.W.G.), on the opposite faces of which are soldered discs B B of copper foil approximately 0·1 mm. thick, as shown in section at (*a*). The centre of one of the discs having been marked by an ink dot, the gas chamber is placed on a block of wood and, by careful tapping, a No. 7 sewing needle is driven squarely through the centres of both discs, so that the point of the needle emerges about 1 mm. from the lower disc, as shown diagrammatically at (*b*). A collar of cork on the needle is useful as an indicator of the correct penetration. The smaller hole, C, is the air jet, the flame issuing from the larger hole D. Gas enters the chamber through hole E, which is about 2 mm. in diameter. To permit attachment of the gas and air lines, two pieces of thin-walled brass tubing F and G (cut from old cork-borers) are soldered to the lower disc as shown, care being taken not to fill up the holes. A bridge of solder H, towards the lower ends of the tubes, renders the assembly completely rigid.

The modification shown in Fig. 2 is for use in the hand instead of in a retort clamp. The gas chamber is formed by bending one end of a length of ⅛-inch outside-diameter copper or brass tubing into a flat loop, as shown at (*a*). Ports A A, for gas entry, are filed, and the chamber is closed by soldering suitably shaped pieces of copper foil to the opposite faces of the loop, as shown in section at (*b*). Holes are pierced through the foils as before and a short length of thin-walled brass tubing B is soldered to the rear of the chamber to allow connection of the air tube. A second length of ⅛-inch outside diameter tubing C, bent as shown, is secured to the gas tube D by two bridges of solder. The upper end of C is connected to B by a short length of cycle valve tubing E. A hook, F, of brass wire enables the blowpipe to be hung up when desired.

The filter-pump-operated device shown in Fig. 3 is a convenient means of producing the necessary air blast. Its construction and principles of operation are similar to those of the stirrer unit previously described.[4] It is, however, larger, and the valve rod has at its lower end a rubber buffer A, of cycle valve tubing, to prevent breakage. A valve chamber B, similar to that previously described,[5] is sealed to the

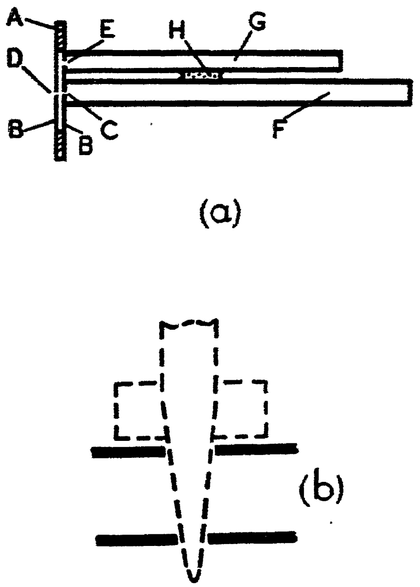

Fig. 1

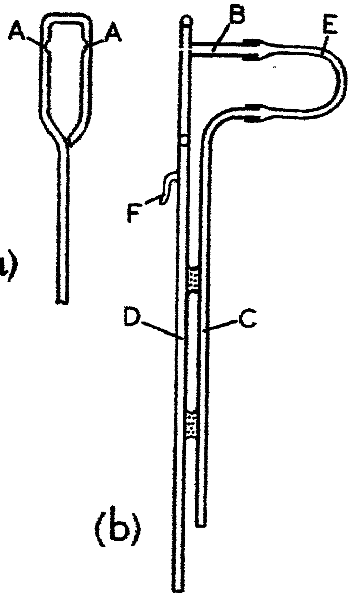

Fig. 2

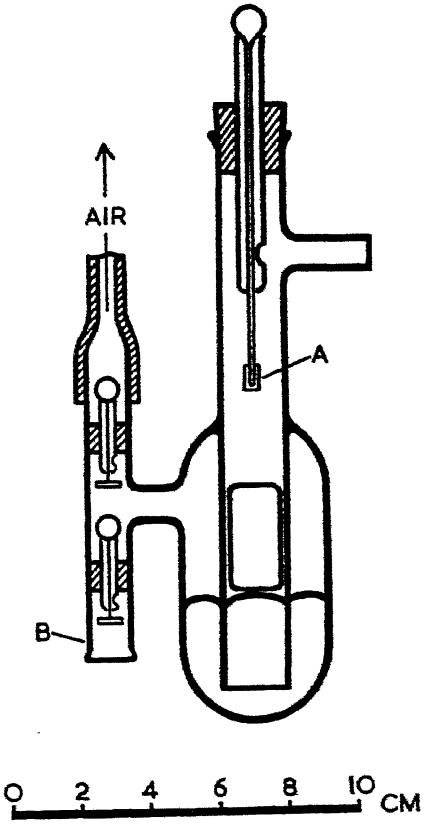

Fig. 3.

outer jacket. Rise and fall of the mercury at about 200 strokes per minute causes air to be drawn in at the lower valve and forced out at the upper one. Insertion of an empty Winchester quart bottle or a 2-litre flask between the pump and the blowpipe gives a steady flame. Suitable adjustment by stopcocks or screwclips gives a hot flame between 3 mm. and 30 mm. in height.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

South African Fish Products. XXVII. Composition of the Liver Oils of the Basking Shark (*Cetorhinus Maximus*, Gunner) and the Spiny Shark (*Echinorhinus Spinosus*, Gmelin). M. L. Karnovsky, W. S. Rapson, H. M. Schwartz and (in part) M. Black and N. J. van Rensberg (*J. Soc. Chem. Ind.*, 1948, **67**, 104–107)—Detailed quantitative study of the saponifiable and unsaponifiable fractions of the liver oils of the basking shark (I) and the spiny shark (II) is described. Results are given for five specimens of I and three of II and for oils from the flesh (tail region), spleen, kidney, epididymia, eye socket, and gristle (underskin) of one basking shark and from the flesh (head region) of another. Unsaponifiable matter of the liver oils of the seven-gilled shark (III) and the souffin shark (IV) was also determined and examined. The hydrocarbons and hydroxylic fractions of the unsaponifiable matter were separated chromatographically by means of activated alumina.

Results—Unsaponifiable matter from I, 30 to 50 per cent., mainly squalene and pristane; from II, about 50 per cent., mainly squalene with some α-glyceryl ethers; from III, about 20 per cent., chiefly α-glyceryl ethers with some sterols; from IV, up to 10 per cent., principally vitamin A, sterols, α-glyceryl ethers, and fatty alcohols. The component fatty acids (weight per cent.) were as shown in the table.

COMPONENT FATTY ACIDS OF THE LIVER OILS OF THE BASKING AND THE SPINY SHARKS, IN WEIGHT PER CENT.

	Basking shark (I)		Spiny shark (II)	
Acids	Saturated	Unsaturated	Saturated	Unsaturated
C_{14}	2·1	0·5 (−2·0 H)	3·9	1·6 (−2·0 H)
C_{16}	13·6	11·9 (−2·0 H)	20·4	11·9 (−2·0 H)
C_{18}	3·2	12·8 (−2·3 H)	6·9	25·6 (−3·0 H)
C_{20}	3·6	23·2 (−4·0 H)	0·3	15·4 (−6·6 H)
C_{22}	3·2	20·0 (−3·6 H)	0·2	13·9 (−8·1 H)
C_{24}	0·4	5·6 (−5·9 H)	—	—

In the relatively low unsaturation of C_{20} and C_{22} acids, oil I resembles other shark liver oils rich in squalene, but the average unsaturation of these acids in II is unexpectedly high and the presence of nearly 6 per cent. of C_{24} acid is also unexpected, as the unsaponifiable matter of II contains much squalene and 13·7 per cent. of α-glyceryl ethers. Oil II therefore does not fit Hilditch's tentative generalisation (Hilditch, "*Chemical Constitution of Natural Fats*," 1941, p. 29). Iodine values given for oils, unsaponifiable matter, and fatty acid indicate that the relationship of high α-glyceryl ether content and lowering of unsaturation of fatty acid also does not hold for basking shark flesh oils.

E. B. DAW

South African Fish Products. XXVIII. Composition of the Liver Oil of the Seven-gilled Shark, *Heptranchias Pectorosus* Garman. M. L. Karnovsky, W. S. Rapson, and H. M. Schwartz (*J. Soc. Chem. Ind.*, 1945, **67**, 144–147)—This liver oil contains a large unsaponifiable fraction, mainly α-glyceryl esters. Characteristics of the oil are: iodine value 118·9, saponification value 159·5, unsaponifiable matter 19·6 per cent., $E^{1\%}_{1\,cm.}$ of oil at 328 mμ. 1·6. The unsaponifiable matter, examined as previously described (*Idem*, *Ibid.*, p. 104), had the following percentage composition: α-glyceryl ethers (as selachyl alcohol) 89·2, unsaturated hydrocarbons (as squalene) 1·1, vitamin A 0·5, and cholesterol 6·4, total, 97·2. The α-glyceryl ethers were freed from sterols and acetylated, the acetates fractionated, and the iodine value, saponification value, and "periodic acid equivalent" determined on each fraction. The component α-glyceryl ethers were calculated as in fatty acid calculations—most fractions being calculated as a mixture of C_n and C_{n+2} saturated and mono-ethenoid components. Re-acetylation and fractionation of the "liquid" glyceryl esters indicated the presence of a considerable amount of an unsaturated α-glyceryl ether acetate of side-chain length less than C_{18}; little saturated matter is present. Evidence for C_{20} and higher fractions is also given. The component fatty acids (weight per cent.) of the saponifiable fraction were:—

	Saturated	Unsaturated
C_{14}	1·6	0·7 (−2·0 H)
C_{16}	16·6	11·0 (−2·0 H)
C_{18}	6·9	30·3 (−2·5 H,
C_{20}	1·3	15·6 (−5·4 H)
C_{22}	1·6	13·0 (−8·7 H)
C_{24}	—	1·4 (−10 H)

The fatty acid composition resembles that of spiny shark oil (*loc. cit.*). The oil does not fit in the tentative generalisation that the presence of large proportions of α-glyceryl ethers in elasmobranch liver oils is associated with marked diminution of polyethenoid unsaturation in C_{20} and C_{22} acids. The relative proportions (mol. per cent.) of fatty acids and α-glyceryl ethers containing alkyl residues with the same number of C atoms were compared as follows:—

		α-Glyceryl ethers in α-glyceryl fraction		Fatty acids in fatty acid fraction	
C_{14}	Saturated	—		2·0	2·9
	Unsaturated	—		0·9	
C_{16}	Saturated	13·9	33·3	18·3	30·6
	Unsaturated	19·4		12·3	
C_{18}	Saturated	15·8	57·4	6·9	37·4
	Unsaturated	41·6		30·5	
C_{20}	Saturated	—	6·2	1·2	15·6
	Unsaturated	6·2		14·4	
C_{22}	Saturated	—	2·5	1·3	12·4
	Unsaturated	2·5		11·1	
C_{24}	Saturated	—	0·6		1·1
	Unsaturated	0·6		1·1	

Experimental details of the treatment of the bulk sample of unsaponifiable matter, of the determination of saponification equivalent (NaOH being substituted for KOH to prevent periodate precipitation later) and "periodic acid equivalent," and of the separation of "solid" and "liquid" compounds are given. Periodic acid equivalents, determined from consumption of periodic acid after saponification, are not up to standard reproducibility of other determinations on these ethers.

E. B. DAW

Polarimetric Determination of Starch in Cereal Products. K. A. Clendenning (*Canad. J. Res.*, 1948, **26**, [F], 185–190)—This paper deals mainly with the particle-size and the extraction-time requirements in calcium chloride polarimetric procedures for starch in whole wheat and wheat flour. Except for wheat gluten, it is not necessary to grind the samples to an impalpable powder as has often been advocated in the past. Wheat, if ground to pass a 40-mesh screen, is sufficiently fine for the polarimetric determination of starch. Excessive grinding may, in fact, cause low results owing to the increase in solubility. In examining the effects of particle-size on starch recovery, the extraction was carried out as usual (*Idem, ibid.*, 1945, [*B*], **23**, 239–259) for periods of 15, 30, and 45 min. Observations were made in 2-dm. tubes, and protein precipitation and filtration were conducted as usual. As the following table shows, a marked improvement in starch recovery was observed only on the very coarsest sample.

TABLE I

EFFECTS OF PARTICLE-SIZE AND EXTRACTION-TIME ON STARCH RECOVERY FROM WHOLE WHEAT AND GRANULAR FLOURS

Sample	Apparent starch content,* % Extracted 15 min.	Extracted 30 min.	Extracted 45 min.
Whole wheat I:			
2-mm. screen	57·84	60·42	60·42
	59·09	60·30	60·52
1-mm. screen	62·09	62·42	62·09
	62·32	62·32	62·32
40-mesh	62·16	61·93	61·60
	62·26	62·04	61·40
ball-milled	61·66	61·46	61·34
	61·66	61·46	61·46
Whole wheat II:			
1-mm. screen	58·74	58·64	58·17
	58·74	58·74	58·17
40-mesh	59·03	59·03	58·60
	58·93	58·82	58·47
ball-milled	58·81	58·81	58·04
	58·93	58·70	58·04
Granular flour:			
as received	76·82	76·82	76·82
	77·14	76·82	76·82
ball-milled	76·84	76·41	76·18
	76·84	76·61	76·28

TABLE II

COMPOSITION OF WHEAT FLOURS, PER CENT., SOLIDS BASIS

	Whole wheat I	Whole wheat II	Granular flour	Patent flour
Ash	1·92	1·87	0·76	0·48
Fat (by acid hydrolysis)	3·82	4·42	1·45	1·58
Protein	15·09	19·52	13·27	15·04
Total sugar	4·10	4·00	2·34	2·18
Pentosan	6·64	7·25	2·91	1·93
Polyuronide	0·78	0·86	trace	trace
Crude fibre	2·63	2·78	0·71	0·18
Starch by difference	64·82	60·30	78·56	78·61
Polarimetric starch (alcohol extraction)	62·16	59·00	77·25	78·5
Polarimetric starch (stannic chloride precipitation) ..	62·20	58·96	76·84	78·2

* Data are expressed on a basis of total solids, corrected for ash increments in milling.

With wheat gluten, extraction for 40 min. was necessary, and a particle-size equivalent to 140-mesh was required for maximum results.

Two polarimetric methods (Eva and Rankin) for starch determination were compared with starch obtained "by difference," and the results, with other analytical data, are shown in Table II.

The difference in crude fat obtainable by various extraction methods is shown below.

TABLE III

COMPARISON OF METHODS FOR THE DETERMINATION OF CRUDE FAT IN WHEAT PRODUCTS, PER CENT.

	Ethyl ether*	Light petroleum*	Acid hydrolysis; ether
Wheat starch ..	0·28	nil	0·48
Wheat flour ..	0·71	0·75	1·60
Whole wheat flour	2·08	1·80	3·00
Gluten (100-mesh)	0·63	0·43	5·00

* By 24-hr. Soxhlet extraction.

W. MARTIN

Biochemical

Separation of Tropic and Atropic Acids by Partition Chromatography. S. Gottlieb (*J. Amer. Chem. Soc.*, 1948, **70**, 423)—These acids are separated by partition between chloroform and water adsorbed on silicic acid; an acid-base indicator is incorporated in the aqueous phase.

Procedure—In a mortar, mix intimately 10 ml. of a 0·1 per cent. aqueous solution of 3 : 6-disulpho-β-naphthalene-azo-N-phenyl-α-naphthylamine with 20 g. of dry precipitated silicic acid. Slurry with 50 ml. of chloroform and pour into a 24-mm. diameter glass tube constricted and plugged with cotton wool at the lower end. Allow chloroform to drain until the suspension is stiff, and pipette into the column the solution of tropic and atropic acids (here, 5 mg. of each in 3 ml. of chloroform). When the solution has just passed the top of the column, wash the sides of the tube with 5 ml. of chloroform. Separate and develop the bands with 50 ml. of chloroform. Atropic acid moves rapidly down in a sharp band; wash it through completely. Wash through the upper band of tropic acid with a mixture of 10 per cent. butyl alcohol in chloroform.

G. H. TWIGG

Estimation of Barbituric Acid and Thiobarbituric Acids in Biological Materials. J. Raventos (*Brit. J. Pharmacol.*, 1946, **1**, 210–214)—A method has been developed for estimating barbituric and thiobarbituric acids present together in the same sample. The main feature is the purification of the extracts containing the acids by chromatography; the estimation of barbituric acids is based on Koppanyi's colour reaction (Koppanyi *et al.*, *J. Amer. Pharm. Assoc.*, 1934, **22**, 1076), whilst thiobarbituric acids are determined by Cowan's colour reaction (demonstration to Physiological Society, 1939, and personal communication).

Preparation of activated alumina—Boil 1200 g. of activated alumina (grade O, Messrs. Peter Spence, Manchester) with 1800 ml. of 10 per cent. acetic acid for 2 hr. Filter and remove the excess of acetic acid by washing with at least 20 litres of hot water. Dry the alumina and heat to 360° C. Cool and add 2·5 per cent. of water to partially deactivate.

Procedure for blood—Mix 10 to 20 ml. volumes of oxalated blood with equal volumes of water and 10 per cent. sodium dihydrogen phosphate solution. Extract with peroxide-free ether in a continuous extraction apparatus at 45° to 50° C. for 8 to 10 hr. Evaporate the ethereal extract to dryness, dissolve the residue in 5 ml. of alcohol-free chloroform, and dry the solution by shaking with 2 g. of anhydrous sodium sulphate. Filter the chloroform solution directly on to an activated alumina column 4 in. high and $\frac{3}{8}$ in. in diameter. Wash the filter three times with 5 ml. of alcohol-free chloroform and pour the washings on to the column. Wash the column with alcohol-free chloroform until the eluate is free from pigment.

Recover the thiobarbituric acids from the column by elution with 50 ml. of 2 per cent. A.R. methanol in chloroform, and then recover the barbituric acids by further elution with 50 ml. of 10 per cent. methanol in chloroform.

Estimation of thiobarbituric acids—Transfer a 2-ml. aliquot of the final chloroform solution to a test tube and add 0·2 ml. of 10 per cent. diethylamine in methanol and 0·5 ml. of a saturated solution of anhydrous copper sulphate in methanol. A green colour develops and is stable for 2 hr. Compare the sample with a series of similarly treated solutions containing 0·03 to 0·5 mg. of the thiobarbituric acid per ml., prepared by dilution of a standard solution containing 0·5 mg. per ml. of the appropriate thiobarbituric acid in chloroform.

Estimation of barbituric acids—For each 2 ml. of the final chloroform solution containing barbituric acids add 0·6 ml. of 5 per cent. *iso*propylamine in methanol and 0·1 ml. of 1 per cent. cobalt acetate in methanol. Compare the red colour given by the sample with a series of similarly treated solutions containing from 0·1 to 1·0 mg. of the appropriate barbituric acid per ml.

Procedure for urine—Add sufficient concentrated hydrochloric acid to the sample to adjust its reaction to *p*H 5. Extract with peroxide-free ether in a continuous extraction apparatus at 45° to 50° C. for 8 to 10 hr. Evaporate the ethereal extract to dryness and dissolve the residue in 5 ml. of alcohol-free chloroform. Continue the determination as described for blood.

Procedure for tissues—Grind 10 to 20 g. of tissue sample with sodium dihydrogen phosphate in the proportion of 1 g. of sodium dihydrogen phosphate to every 10 g. of tissue. Add slowly 20 g. of anhydrous sodium sulphate for each 10 g. of tissue and grind the mixture to a fine powder. Transfer the mixture to a desiccator and allow to stand over anhydrous calcium chloride for 1 hr. Extract the powder for 2 to 3 hr. with 50 ml. of A.R. benzene in a flask: filter the extract and wash the flask and residue three times with 15 ml. of benzene. Mix the filtrate and washings and concentrate to 5 ml. by heating at 50° C. under reduced pressure.

Pour the concentrated benzene solution on to an alumina column, wash the flask three times with benzene, and pour the washings through the column. Wash the column with benzene until the eluate is free from pigment and then wash with 20 ml. of chloroform.

Recover and determine the barbituric and thiobarbituric acids by the method described for blood.

Recoveries of barbituric and thiobarbituric acids average 97·3 per cent. A. H. A. Abbott

Water Analysis

Water Analyses by Selective Specific Conductance. J. W. Polsky (*Anal. Chem.*, 1947, **19**, 657–660)—A simple and rapid method is described for the determination of individual ions in dilute solution, with particular reference to the determination of chloride, sulphate, and calcium in water.

The method involves the measurement of specific conductivity of a sample solution before and after addition of an excess of the appropriate reagent to precipitate the ion being determined. The effect of the reagent addition is to increase the specific conductivity, whilst a decrease is caused by the precipitation taking place. If the amount of reagent added is kept constant, the observed change in specific conductivity can be correlated with the amount of ion being determined.

For the dilute solutions dealt with in water analysis, where the activity of the ions approaches unity, the change in specific conductivity due to addition of a fixed quantity of reagent is virtually independent of the nature of the ions present, provided that the initial specific conductivity of the solutions is the same. For solutions of different initial specific conductivities the change due to the addition of the reagent decreases with increasing initial specific conductivities, owing to lower ionic mobilities at the higher concentrations, and a correction that depends upon the initial specific conductivity must be applied.

The method is of general application providing that the solutions employed are dilute. The procedure for determination of chloride serves to illustrate the technique.

Preparation of standard chloride and conductivity correction curves—Prepare 100-ml. samples of standard sodium chloride solutions to cover the range 0 to 80 p.p.m. of chloride. Pour the first standard into a suitable cylinder, adjust to the desired standard temperature and measure the conductivity with a dipping-type conductivity cell. Transfer the solution to a beaker and, with stirring, pipette in 10 ml. of standard silver nitrate solution (1 ml. ≡ 1 mg. of chloride). Return the solution to the cylinder and again measure the conductivity. Repeat the procedure with the other standards, recording the initial specific conductivity and the change in specific conductivity for each chloride concentration.

To determine the correction to be applied for the initial specific conductivity of the sample, prepare a series of 100-ml. sodium sulphate solutions having a range of specific conductivities of 2 to 700 reciprocal megohms per cm. per cm.[2] Measure the conductivities as above before and after the addition of 10 ml. of standard silver nitrate solution, recording the change in specific conductivity for each initial specific conductivity. To obtain the conductivity correction for each initial specific conductivity, subtract the change in specific conductivity from the change observed for the solution having an initial specific conductivity of 2 reciprocal megohms. Prepare a graph of conductivity correction against initial specific conductivity.

Apply the conductivity correction to the figure for change in specific conductivity obtained for each chloride standard by adding the conductivity correction corresponding to the initial specific conductivity of the chloride standard. Prepare a graph of the corrected values for change in specific conductivity against chloride concentration.

Determination of chloride—Using 100 ml. of sample, determine the specific conductivity before and after addition of 10 ml. of standard silver nitrate solution. To the observed change in specific conductivity add the conductivity correction corresponding to the initial specific conductivity of the sample and, using this corrected value, read off from the standard chloride curve the chloride concentration of the sample.

Note—The *p*H of a sample should be observed before starting a determination and, if necessary, addition of acid or alkali should be made to adjust the *p*H to the range most favourable for precipitation of the ion to be determined. The "initial" specific conductivity is then measured and the determination continued in the normal manner. H. J. Cluley

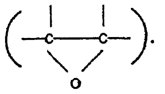
Gas Analysis

Estimation of Sulphur Trioxide in its Mixtures with Sulphur Dioxide by the Method of Amperometric Titration. D. W. E. Axford and T. M. Sugden (*J. Chem. Soc.*, 1946, 901–903)—An amperometric titration is described for determining sulphur trioxide in the presence of a large excess of sulphur dioxide. The gases are absorbed in sodium hydroxide solution and, after acidification, the sulphur dioxide is blown out by a stream of nitrogen. The sulphate is then titrated with a standard solution of lead nitrate.

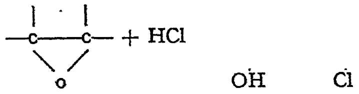
Procedure—Pass the gas mixture through 1·0 *N* sodium hydroxide to absorb the sulphur trioxide and sulphur dioxide. The sodium hydroxide should contain a few drops of glycerol to inhibit the oxidation of sulphur dioxide. Pipette 5 ml. of the solution into a suitable polarographic cell and add 1·0 *N* nitric acid until the solution is acid to methyl orange. After passing a stream of nitrogen through the solution for 30 min. to drive off the sulphur dioxide, add 1·0 *N* sodium hydroxide until the solution is just alkaline to methyl orange, followed by 5 ml. of ethyl alcohol to reduce the solubility of the lead sulphate and a small amount of solid lead sulphate to prevent supersaturation. Set the potential of the dropping mercury electrode at −0·8 v. *versus* the mercury pool and titrate the solution with 0·01 *N* lead nitrate, plotting the current flowing against the volume added. The end-point can be read off from the graph with an accuracy of within ±2 per cent. J. G. WALLER

Organic

Determination of Oxirane Oxygen. D. Swern, T. W. Findley, G. N. Billen, and J. T. Scanlan (*Anal. Chem.*, 1947, **19**, 414–415)—Although in the study of atmospheric oxidation of unsaturated fatty materials it is customary to determine the quantity of hydroxyl, carboxyl, carbonyl, and ester oxygen, no work has been reported on the analysis of oxirane oxygen



Nicolet and Poulter (*J. Amer. Chem. Soc.*, 1930, **52**, 1186) devised a method for the quantitative opening of the oxirane ring, by means of a solution of anhydrous hydrogen chloride in absolute ethyl ether, for use in the analysis of 9 : 10-epoxystearic acids. The equation



suggests that the method might be specific for oxirane oxygen, and the present authors have examined compounds with and without the group. They conclude that only oxirane oxygen reacts with this reagent; and, by modification of the concentration and the reaction period, the method has been made suitable for use in the analysis of the unknown and complex mixtures encountered in atmospheric oxidation reactions.

Special reagent—Add approximately 25 to 35 ml. of concentrated hydrochloric acid dropwise during about 45 min. to concentrated sulphuric acid. Pass the anhydrous hydrogen chloride into 1500 ml. of absolute ethyl ether until a blank determination indicates that the concentration is 0·19 to 0·20 *N*. To conduct this determination pipette 25 ml. of the solution into 50 ml. of 95 per cent. ethyl alcohol and titrate with 0·1 *N* sodium hydroxide, using a 1 per cent. solution of phenolphthalein in 95 per cent. ethyl alcohol as indicator. Store the reagent in glass-stoppered bottles.

Procedure—Weigh an aliquot of the sample in accordance with the recommendations in Table I, and transfer to a 250-ml. flask fitted with a hollow glass stopper. (Because of its light weight the hollow stopper acts as an automatic safety valve and makes the manual release of pressure unnecessary.) Wash the sides of the flask with 5 ml. of ethyl ether and add 25 ml. of the hydrochloric acid - ether reagent. Stopper the flask, swirl gently to dissolve the sample, and allow to stand for 3 hr. at room temperature. Add 50 ml. of 95 per cent. ethyl alcohol and 1 ml. of a 1 per cent. solution of phenolphthalein in 95 per cent. ethyl alcohol and titrate the excess of acid with 0·1 *N* sodium hydroxide. Make at least two blank determinations, which must agree within 0·1 ml.

TABLE I

RECOMMENDED SAMPLE WEIGHTS FOR OXIRANE OXYGEN DETERMINATION

Oxirane oxygen expected, %	Weight of sample, g.
1 to 4	1·0 to 0·8
4 to 8	0·8 to 0·4
8 to 12	0·4 to 0·25
12 to 16	0·25 to 0·20
16 to 20	0·20 to 0·15

Weigh a second sample of from 0·3 to 2·0 g., dissolve it in 75 ml. of freshly neutralised 95 per cent. ethyl alcohol, and titrate to the phenolphthalein end-point with 0·1 *N* sodium hydroxide. For oils insoluble in alcohol, heat the sample under reflux with the alcohol for 5 min., cool to room temperature, and titrate with 0·1 *N* sodium hydroxide.

Calculate the oxirane oxygen from the expression

$$\text{Percentage of oxirane oxygen} = \frac{B-(T-A)\times 1{\cdot}6}{W}$$

in which B = ml. of 0·1 *N* sodium hydroxide used in the blank; T = ml. of 0·1 *N* sodium hydroxide used in the back-titration; A = acid value of sample expressed as ml. of 0·1 *N* sodium hydroxide used to neutralise the free acid in W g. of the sample; W = weight of sample in grams.

Table II shows the results obtained when the procedure described above was applied to a selection of purified oxirane compounds. The anomalous result obtained with styrene oxide may be due to the formation of an isomeric aldehyde or ketone by acid catalysis.

TABLE II

ANALYSES OF PURIFIED OXIRANE COMPOUNDS

Compounds analysed	Per cent. of oxirane oxygen	
	Calcd.	Found
9 : 10-Epoxystearic acid, m.p. 59·5° C.	5·36	5·33
9 : 10-Epoxystearic acid, m.p. 55° C.	5·36	5·35
9 : 10-Epoxyoctadecanol, m.p. 54° C.	5·62	5·65
9 : 10-Epoxyoctadecanol, m.p. 48° C.	5·62	5·60
9 : 10 : 12 : 13-Diepoxystearic acid, m.p. 78° C.	10·2	9·16
Methyl 9 : 10-epoxystearate, m.p. 16° C.	5·12	5·08
Methyl 10 : 11-epoxyhendecanoate	7·47	7·28
Epichlorohydrin	17·3	16·8
Glycidol	12·6	12·3
1 2-Epoxyoctane	12·5	12·0
1 2-Epoxydecane	10·2	10·0
1 2-Epoxydodecane	8·68	8·53
1 2-Epoxytetradecane	7·54	7·53
1-Phenyl-1 : 2-epoxyethane (styrene oxide)	13·3	11·7

A. H. A. ABBOTT

Determination of Sulphur in Bone Char. V. R. Deitz, H. R. Higginson, and C. Parker (*J. Res. Nat. Bur. Stand.*, **1948**, **40**, 263–274)—The determination of sulphate sulphur by treatment of bone char with hydrochloric acid, and of total sulphur by treatment with nitric acid, using established procedures, gives inaccurate results owing to the presence of organic sulphur. The barium sulphate precipitate can be freed from calcium and from phosphate by dissolution in 70 per cent. perchloric acid and re-precipitation.

Method 1—*Total sulphur*—Treat 5 g. of bone char, dried at 105° to 110° C. for at least 4 hr., in a 800-ml. beaker with 10 to 15 ml. of water and 25 ml. of concentrated nitric acid. Boil gently for 1·5 hr., add 15 ml. of 70 per cent. perchloric acid (stage B) and heat until the char is completely oxidised. Cool, dilute with 100 ml. of water, and filter through a No. 40 Whatman paper (stage A) into a 1500-ml. beaker. Wash the residue with 150 to 200 ml. of water, dilute to 750 to 800 ml. and adjust the *p*H to 1 (glass electrode) with aqueous ammonia. Heat to 50° C., add 50 ml. of 5 per cent. barium chloride solution dropwise with mechanical stirring, keep at 50° C. for 4 hr., and filter through a No. 42 paper. Wash until only a trace of chloride is detectable. Transfer paper and precipitate to a 800-ml. beaker, add 25 ml. of nitric acid, warm to destroy the paper, add 15 ml. of perchloric acid, and continue boiling for 15 min. after the nitric fumes have ceased, to give a colourless or slightly yellow solution. Cool to room temperature, add 400 ml. of water and 5 ml. of 5 per cent. barium chloride solution, and adjust the *p*H to 1. Leave to stand overnight, filter, wash, ignite, and weigh.

Method 2—*Sulphur in the filtrate from acid leaching*—Add slowly 100 millilitres of diluted hydrochloric acid (1 + 1) to 10 grams of the bone char wetted with 25 ml. of water in a 800-ml. beaker. Heat under a cover for 1·5 hr. at 80° to 90° C. Cool, dilute accurately in a 250-ml. calibrated flask, and filter exactly 200 ml. through a No. 40 paper into a 200-ml. calibrated flask. Determine the inorganic sulphate in this filtrate as in Method 1, starting at stage A. Multiply the result by $(250 - w/1{\cdot}8)/200$, where w is the dry weight in grams and 1·8 is the density of the carbonaceous residue.

Method 3—*Combined organic sulphur*—To the remaining filtrate in the 250-ml. flask, the residue, and the filter paper obtained in Method 2, add 25 ml. of nitric acid and heat until the paper is decomposed. Add 15 ml. of perchloric acid and determine the sulphate as in Method 1 from stage B. Subtract from the result the sulphur figure, obtained by Method 2, multiplied by $(50 - w/1{\cdot}8)/(250 - w/1{\cdot}8)$.

Method 4—*Sulphide sulphur*—Connect to an Erlenmeyer flask with a ground-glass joint a low-pressure air supply, a dropping funnel and, through a reflux condenser, two fritted-disc gas-washing bottles in series. Place in the gas-washing bottles a solution obtained by diluting to 100 ml. one millilitre of a solution containing 168 g. of cadmium sulphate, 960 ml. of concentrated aqueous ammonia, and 1046 ml. of water. Cool the first bottle in ice. Place 5 g. of the bone char in the flask and run in slowly 50 ml. of diluted hydrochloric acid (1 + 1). After the first reaction sweep out with air and heat gently for 20 min. Add 25 ml. of concentrated hydrochloric acid to the wash bottle solution and titrate with iodine, using starch as indicator. Subtract the value given by a blank determination.

C. F. Herbert

Identification of Organic Acids and Nitrophenols by their Benzylammonium salts. R. Boudet (*Bull. Soc. Chim.*, **1948**, 390–392M)—The methods of preparation are designed to avoid contamination of the salts with the carbonate of the base. Drying by long exposure in the air is avoided because the salts are somewhat hygroscopic. The failure of Buchler *et al.* (*J. Amer. Chem. Soc.*, 1935, 57, 2181) to observe these precautions is the probable reason for numerous discrepancies between the m.p. given by them and those now determined. The neutral salts of polybasic acids are more readily purified than the acid salts and the former are therefore prepared.

Procedure for monobasic acids and nitrophenols—Take 1 to 3 g. of the acid according to the molecular weight. If the molecular weight is unknown, take 3 g. to ensure an excess. Dissolve this sample in benzene, with addition of a small amount of alcohol if necessary, and add 2 ml. of a water-free mixture of equal volumes of benzylamine and benzene. Agitate the mixture vigorously and allow to cool and crystallise. If it is not possible to induce crystallisation by ordinary means, the solution can be diluted with light petroleum, but there is then a risk of precipitating the excess of acid. Alternatively, the salt can be extracted with water, the aqueous solution washed with ether to remove free acid, and the derivative obtained by evaporation to dryness on a water-bath. Wash the filtered salt with a solvent that dissolves the free acid selectively, such as benzene or benzene - alcohol or benzene - light petroleum mixtures. Recrystallise from carbon tetrachloride mixed, if necessary, with the minimum quantity of alcohol, filter at the pump, leave on the pump for about 15 min. and determine the melting-point.

Procedure for polybasic acids—Dissolve 0·2 to 0·3 g. of the acid in the minimum quantity of alcohol, add 1 ml. of benzylamine, and shake. After a few minutes, redissolve the precipitated salt by boiling, adding a little more alcohol if necessary. On cooling, the salt crystallises, any carbonate formed remaining in solution. Filter at the pump, wash with a little alcohol, leave on the pump for about 15 min., and determine the m.p.

The following are the m.p. (in ° C.) of the salts of the acids named: *n*-butyric, 54–55*; *n*-valeric, 56–57; propionic, 68; lauric, 70–73·5*; stearic, 70·3–70·5; caproic, 71·5–72; oenanthic, 73·2–73·8; *iso*valeric, 74–76*; *cyclo*propane-carboxylic, 74·5–75; caprylic, 75–76; myristic, 77–78; palmitic, 79–79·5; *iso*butyric, 92–93; 4-chlorocrotonic (*trans*), 93·5–94·5; acetic, 97·5–98·5; 2-hydroxy-3 : 4-dibromobutyric, 100–101*; formic, 100–101; *p*-nitrophenol,

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* Bad m.p.

103–103·5; dichloroacetic, 105–105·5; trichloroacetic, 118–119 (decomp.); anthranilic, 113–114; 2-chlorocrotonic (*trans*), 114–115; chloroacetic, 120–121·2; *o*-nitrophenol, 121–121·5; sebacic, 121·5–122·5; phenylacetic, 122–123; citraconic, 125–125·5; malic, 126·5–127; benzoic, 129–129·5; cyanoacetic, 131–131·5 (decomp.); itaconic, 132–133; cinnamic, 131–131·5; malonic, 142·5–143; anisic, 143·5–144·5; *o*-nitrobenzoic, 147–148·5; succinic, 147·5–148; 2 : 5-dinitrophenol, 147·5–148; tartaric, 147·5–148; 2-ethoxymalonic, 155–155·5 (decomp.); 1-cyano-*cyclo*propane-1-carboxylic, 156·5–157·5; adipic, 162·5–163·5; *p*-toluic, 164–164·5; 2 : 6-dinitrophenol, 166–167; tartronic, 170–172; fumaric, 171–172; 2 : 4-dinitrophenol, 171·3–171·5; *cyclo*-propane-1 : 1-dicarboxylic, 173–173·5 (decomp.); *m*-nitrobenzoic, 177–178; camphoric, 179–181; fumaric, 181–182; phthalic, 184·5–185; gallic, 188–190; trinitrophenol, 195–196; oxalic, 195–196; *p*-nitrobenzoic, 198–199; 2-chloro-3 : 4-dibromobutyric, 200–205.*

The salt of mucic acid, m.p. 200° C., is prepared by mixing an excess of the acid with benzylamine and extracting the mixture with cold dilute alcohol.

It is generally possible to prepare the benzylamide by heating the benzylammonium salt for 1 hr. in the air. The m.p. of the benzylamide can than be determined as a confirmatory test (Dermer and King, *J. Org. Chem.*, 1943, 8, 168).

W. C. Johnson

Titration of Acyl Chlorides. M. Pesez and R. Willemart (*Bull. Soc. Chim.*, 1948, 479–480M)—The method uses the reaction represented by the following equation:

$$R.COCl + NH_2C_6H_5 \rightarrow HCl + R.CO.NHC_6H_5.$$

Any free organic acid present does not enter into this reaction and is subsequently titrated, together with the hydrochloric acid liberated. Further titrations of the total acid and of the chloride, after hydrolysis, provide the necessary data for calculating the constitution of the sample. Dioxan is used as the solvent by reason of its miscibility with water, its good solvent properties for organic acids and acid chlorides, and the ease with which it is obtained free from water and alcohol by rectification over sodium.

Procedure—(1)—Dissolve a quantity, p g., of the sample, equivalent to 10 to 15 ml. of 0·1 N silver nitrate, in 15 ml. of dioxan, add 10 ml. of water, and titrate with 0·1 N sodium hydroxide to the green colour of bromothymol blue. Volume of 0·1 N sodium hydroxide required = a ml. (2)—To the same solution add 5 ml. of diluted nitric acid (1 in 5), 20 ml. of 0·1 N silver nitrate, and 10 drops of a saturated, aqueous solution of potassium permanganate. Decolorise by adding a few drops of 3 per cent. hydrogen peroxide, free from halogens, and titrate with 0·1 N potassium thiocyanate, using ferric alum as indicator. Volume of 0·1 N silver nitrate equivalent to chloride present = c ml. (3)—Dissolve a further quantity, p' g., of the acid chloride in 10 ml. of dioxan and add 2 ml. of freshly distilled aniline. Stir for a few seconds, add 100 ml. of water, and titrate with 0·1 N sodium hydroxide, using bromothymol blue as indicator. Volume of 0·1 N sodium hydroxide required = b ml.

If M = molecular weight of the acid chloride and m = that of the organic acid, then:

Acid chloride, per cent. =

$$\left(\frac{a}{p} - \frac{b}{p'}\right) \times \frac{100\ M}{10{,}000}$$

Organic acid, per cent. =

$$\left(\frac{b}{p'} - \frac{c}{p}\right) \times \frac{100\ m}{10{,}000}$$

Free hydrochloric acid, per cent. =

$$\left(\frac{c - a}{p} + \frac{b}{p'}\right) \times \frac{36{\cdot}5 \times 100}{10{,}000}$$

The method is applied to the analysis of acetyl chloride, benzoyl chloride, and its *p*-nitro- and 3 : 5-dinitro- derivatives, and to the chloride of acetylsalicylic acid.

W. C. Johnson

(A) Aldehydes and the Doebner - Miller Reaction. (1) 5-Carboxy-7-aminoquinaldine. (2) Detection of the –CH_2.CHO Group. L. Velluz, G. Amiard, and M. Pesez. (3) Estimation of Acetaldehyde. L. Velluz, M. Pesez, and M. Herbain. (B) A New Fluorescence Indicator. L. Velluz and M. Pesez (*Bull. Soc. Chim.*, 1948, 678–679M, 680–681M, 681–682M, 682–683M)—(A) (1)—von Bitto (*Z. anal. Chem.*, 1897, **36**, 369) found that strongly fluorescent substances are produced by the reaction of *m*-phenylenediamine salts with aldehydes, but he isolated no definite compounds. The analogous product from the condensation of 3 : 5-diaminobenzoic acid with acetaldehyde is now prepared, and is shown to be 5-carboxy-7-aminoquinaldine. This substance also exhibits a green fluorescence in acid solutions.

Preparation of 5-carboxy-7-aminoquinaldine—To an ice-cold mixture of 10 ml. of paraldehyde and 10 ml. of ethyl alcohol slowly add 50 ml. of a 50 per cent. aqueous solution of 3 : 5-diaminobenzoic acid dihydrochloride previously cooled to 0° C. After keeping for several hours at 15° to 17° C., filter off the yellow needles of 5-carboxy-7-aminoquinaldine hydrochloride and wash them with water. The water decomposes the hydrochloride; 4 g. of the amino acid are obtained. Dissolve the product in 5 volumes of 1·0 N sodium hydroxide, add charcoal, filter, and reprecipitate with carbon dioxide. Filter off, wash with water, and dry at 100° C. The product contains 1 mol. of water of crystallisation. Alternatively, the crude product may be purified by recrystallisation from 60 volumes of boiling acetic acid followed by the above process of reprecipitation to obtain the hydrate.

(2) The reaction already described does not occur with the common ketones and is confined to aldehydes that contain an α-methylene group. It may be used as a sensitive test for acetaldehyde and thus, indirectly, for a number of other substances such as ethyl alcohol and lactic acid.

Procedure—Dissolve 2 g. of 3 : 5-diaminobenzoic acid hydrochloride in 50 ml. of phosphoric acid

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* Bad m.p.

(density 1·62) and dilute to 100 ml. with water. Decolorise, if necessary, with charcoal. Add to the aldehyde solution an equal volume of the above reagent. A yellow colour and a yellow-green fluorescence develop more or less rapidly according to the concentration of aldehyde. For concentrations of acetaldehyde of the order of 10^{-4} the fluorescence is directly perceptible after 15 min. heating on a water-bath. Concentrations down to 2×10^{-6} may be detected, after several hours standing, by the use of an ultra-violet lamp.

m-Phenylenediamine hydrochloride can be used in place of the diaminobenzoic acid hydrochloride. The sensitivity for acetaldehyde is then 3×10^{-5} after 1 hr. and 2×10^{-5} after 24 hr.

(3) The intensity of the fluorescence of 5-carboxy-7-aminoquinaldine varies with the acidity of the solution, becoming less intense in solutions of low *p*H. By the use of phosphoric acid these variations are minimised and the fluorescence intensity is a linear function of the acetaldehyde concentration between 10^{-4} and 10^{-6}.

Reagent—Prepare an aqueous solution containing 10 g. of 3 : 5-diaminobenzoic acid dihydrochloride in 100 ml. and filter through "Acticarbone," if necessary, to remove any fluorescent impurities.

Standard—Dissolve 50 mg. of 5-carboxy-7-aminoquinaldine in 1 litre of a 5 per cent. solution of hydrochloric acid. To 2 ml. of this solution add 10 ml. of 7 *M* phosphoric acid and dilute with water to 100 ml. The parent solution is stable in the dark, but the diluted standard (1 ml. = 1 μg. of carboxy-aminoquinaldine) should be renewed after each determination.

Fluorimetric procedure—To 3 ml. of a solution containing 10 to 100 μg. of acetaldehyde add 2 ml. of the reagent and 5 ml. of 7 *M* phosphoric acid, heat over boiling water for 10 min. and leave in a water-bath at 15° to 17° C. for 2 hr. Add 5 ml. of 7 *M* phosphoric acid and dilute with water to 20 ml. Determine the fluorescence intensity with reference to that of the standard, using the Meunier electrophotometer with a green filter.

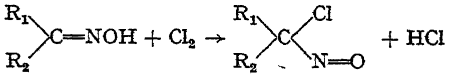
(B) The fluorescence of an acid solution of 5-carboxy-7-aminoquinaldine disappears when such a solution is made alkaline, but the change is not well marked with respect to any definite *p*H value. The N-acetyl derivative of the same substance shows a bright violet-blue fluorescence, which fades sharply over the narrow range *p*H 7·6 to 8. The acetyl compound may, therefore, be employed as a fluorescence indicator in the titration of weak acids.

Preparation of 5-carboxy-7-acetylaminoquinaldine—Heat under a reflux condenser for 1 hr. a suspension of 5-carboxy-7-aminoquinaldine in 10 ml. of acetic anhydride. Cool, add 50 ml. of water, allow to react, and partly neutralise with sodium hydroxide. Recrystallise the precipitated acetyl derivative from 250 ml. of boiling water, decolorising with charcoal. M.p. 315° C. (decomp.).

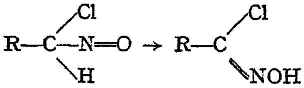
Prepare a solution of 0·1 g. in 1 litre of 50 per cent. ethyl alcohol and use 0·1 ml. of this solution in acidimetric titrations. For coloured solutions, 1 ml. of a 0·05 per cent. solution is recommended.

W. C. JOHNSON

Detection and Colorimetric Estimation of Oximes, Ketones, and Hydroxylamine. O. Wichterle and M. Hudlicky (*Coll. Czech. Chem. Comm.*, 1947, **12**, 661–670)—Chlorine and other chlorinating agents convert oximes into intensely blue chloronitroso compounds (Piloty and Stock, *Ber.*, 1902, **35**, 3093).



Those derived from aldoximes rapidly rearrange to colourless hydroxamic acid chlorides,



but the ketoximes yield chloronitroso derivatives sufficiently stable for colorimetric estimation. The reaction can be employed qualitatively and quantitatively for ketones that readily form oximes, and for hydroxylamine. *Cyclo*hexanone oxime, for example, is detectable at a dilution of 1 : 10,000 in 10 ml.

Preparation of chlorinating agent—Moisten 60 g. of urea with 15 ml. of water, cool below 0° C., and pass in chlorine until the weight has increased by 30 to 32 g. Remove the crystals. The syrupy liquid decomposes slowly, but is usable for several weeks as it is employed in considerable excess.

Detection of oximes—To a solution of the substance in an organic solvent add several drops of the chlorinating agent. The presence of an oxime is shown by a blue colour. If the sample is dissolved in water the blue substance may precipitate and extraction with an organic solvent is then desirable.

Detection of ketones—Add an excess of a solution containing 8·2 g. of hydroxylamine sulphate and 13·7 g. of crystalline sodium acetate in 1 litre of water and proceed as in the previous paragraph.

Detection of hydroxylamine—Neutralise any mineral acid with sodium acetate, add *cyclo*pentanone and a few drops of chlorinating agent and shake with xylene.

The same principles are applied to the colorimetric estimation of *cyclo*hexanone oxime, *cyclo*pentanone oxime, *cyclo*hexanone, and hydroxylamine. The most suitable solvent is glacial acetic acid. If the reaction is carried out in dilute acetic acid the results are not reproducible, but once the colour is formed in the concentrated acid, dilution has no effect, except to precipitate the chloronitroso compound if carried to excess. The extinction of a 0·01 *M* solution of chloronitroso*cyclo*hexane decreases by 2 per cent. per hour, but this does not affect significantly determinations carried out with normal dispatch. The extinction curves for 1-chloro-1-nitroso*cyclo*hexane and 1-chloro-1-nitroso*cyclo*pentane, determined in acetic acid solution with a Lange universal colorimeter and a Soudan 5B filter, employing the deviation method, are linear for concentrations up to 0·01 *M*. These characteristics are determined on samples of the pure chloronitroso compounds, the preparation of which is described. When using the method for the control of the manufacture of ϵ-caprolactam from *cyclo*hexanone oxime the presence of the lactam leads to slightly high results, and the presence of sulphuric acid to low results. Sulphuric acid must therefore be neutralised with a solution of fused sodium acetate in glacial acetic acid.

Estimation of cyclo*hexanone oxime*—Dissolve the sample containing 0·01 to 0·06 g. of the oxime in 10 ml. of acetic acid and add 0·2 to 0·3 ml. of an aqueous solution of chlorinated urea. Add 30 ml. of water and dilute to 50 ml. with acetic acid. Measure the extinction of the solution and read the concentration from a calibration graph. The graph should be checked frequently. The error is ±2 per cent. for 0·005 to 0·01 *M* solutions, but for less than 5 mg. per 50 ml. the error may be 30 per cent.

Estimation of cyclo*pentanone oxime*—Dissolve the sample containing 0·01 to 0·05 g. of the oxime in 10 ml. of acetic acid, add 0·25 ml. of chlorinating agent and 25 ml. of water, and dilute to 50 ml. with acetic acid. The solutions used for calibration must also contain 50 per cent. of acetic acid since the acid concentration affects the colour intensity of chloronitrosopentane solutions.

Estimation of cyclo*hexanone*—To the sample containing 0·01 to 0·05 g. of the ketone add 3 ml. of 0·5 *M* hydroxylamine acetate. After 5 to 10 min., add 20 ml. of acetic acid and 0·5 ml. of chlorinating agent, and dilute to 50 ml. with water. The calibration curve prepared for the oxime serves also for the ketone.

Estimation of hydroxylamine—Dissolve the sample containing the equivalent of 0·01 to 0·035 g. of the hydrochloride in 1 ml. of 0·2 *M* sodium acetate, add 0·1 ml. of *cyclo*hexanone, 20 ml. of acetic acid, and 0·25 ml. of chlorinating agent, and dilute to 50 ml. with water. *Alternatively*, the estimation may be carried out in aqueous solution if a keto acid is used in place of *cyclo*hexanone. Dissolve the sample containing 0·01 to 0·04 g. of the hydroxylamine salt in 5 ml. of water and add 3 *N* sodium hydroxide to the colour change of phenolphthalein; add 2 ml. of a 0·5 *M* solution of *cyclo*hexanone-3 : 4-dicarboxylic acid and, after 2 to 5 min., 0·5 to 1 ml. of chlorinating agent, and dilute to 50 ml. with water. The calibration curve is constructed with the use of reagent hydroxylamine hydrochloride.

W. C. JOHNSON

Analysis of Aliphatic Amine Mixtures. Determination of Tertiary Amines in the Presence of Primary and Secondary Amines and Ammonia. C. D. Wagner, R. H. Brown, and E. D. Peters (*J. Amer. Chem. Soc.*, 1947, **69**, 2609–2610)—The method of Blumrich and Bandel (*Angew. Chem.*, 1941, **54**, 374) consists of treatment with acetic anhydride and titration of the unacetylated tertiary amine with perchloric acid in acetic acid. The method is now shown to be capable of general application except to mixtures containing secondary amines in which the nitrogen is attached to secondary alkyl groups. More vigorous conditions are prescribed for such sterically-hindered substances. When these conditions are adopted the presence of some acetic acid is necessary to prevent significant decomposition of the tertiary amine.

Reagent and apparatus—Prepare 0·1 *N* perchloric acid by mixing the appropriate amount of the 70 per cent. acid with glacial acetic acid, and standardise against anhydrous sodium carbonate dissolved in acetic acid, titrating potentiometrically or to the crystal-violet end-point. For the potentiometric methods the authors used glass and calomel electrodes with a direct reading Beckman Model M or Precision-Shell (Penther and Rolfson, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 337) titrometer. In the examples quoted the end-point inflections in the titration curves are in the region of 300 to 400 millivolts electrode potential.

General procedure—Add not more than 2 g. of the sample, containing not more than 1 g. of water, to 20 ml. of acetic anhydride containing 2 ml. of acetic acid, and allow the mixture to stand for 3 hr. at room temperature. Then add 30 ml. of acetic acid and titrate the mixture potentiometrically with 0·1 *N* perchloric acid in acetic acid.

Special procedure for sterically-hindered secondary amines—The method is similar to the general procedure except that the mixture is "gently refluxed" for 1 hr. instead of being kept at room temperature for 3 hr.

In presence of large amounts of amides the end-point is often slightly buffered, but when the sample consists of essentially pure tertiary amine the end-point is sharp and crystal violet can be used as a colorimetric indicator in place of the potentiometric procedure. The use of the indicator in the analysis of any particular mixture, however, should first be investigated by parallel experiments using the indicator and the potentiometric method on a typical sample.

W. C. JOHNSON

Analysis of Aliphatic Amine Mixtures: Determination of Secondary plus Tertiary Amines by the Azomethine-Acidimetric Method. C. D. Wagner, R. H. Brown, and E. D. Peters (*J. Amer. Chem. Soc.*, 1947, **69**, 2611–2614)—If an aromatic aldehyde is added to a mixture of a primary and a secondary amine and the mixture titrated potentiometrically in a non-aqueous solvent, a sharp end-point corresponding to the neutralisation of the secondary amine is observed. The azomethine, formed by reaction of the primary amine with the aldehyde, is a much weaker base and gives rise to a less distinct end-point. The primary amine may be assayed by this second end-point but is better determined by difference after titration of the total bases. Ammonia, in absence of water, titrates as primary amine but, when water is present, interferes with the secondary amine end-point and must be separated. In the procedure recommended (*infra*) quantities of ammonia down to 0·37 mg.-equivalents cause significant error when 5 g. of water are also present. A method for the separation and determination of ammonia is described. When ammonia is absent the sample of amines may contain as much as 95 per cent. of water. Tertiary amines, when present, do not react with the aldehyde and are determined with the secondary amines. They may be determined separately by the method described in the preceding abstract.

Reagents—(1) Hydrochloric acid, 0·5 *N*, in *iso*propyl alcohol. Mix one volume of concentrated hydrochloric acid with *iso*propyl alcohol to give a total of 24 volumes and standardise against a standard alkali. (2) Salicylaldehyde free from salicylic acid. (3) Sodium cobaltinitrite solution. Dissolve 25 g. of pure cobalt nitrite and 25 g. of sodium nitrite in water and dilute to 200 ml.

Apparatus—The authors recommend the Beckman Model M or the Precision Shell (Penther and Rolfson, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 337) direct-reading titrometer with glass and calomel electrodes and a 10-ml. burette, reading to 0·05 ml.

Procedure—Add the sample, containing about 4 mg.-equivalents of secondary amine, to 80 ml. of methanol and 5 ml. of salicylaldehyde. Mix, cover with a watch-glass, and allow to stand for 30 min. Titrate potentiometrically with 0·5 *N* hydrochloric acid in *iso*propyl alcohol. Calculate the secondary (plus tertiary) amine as equivalent to the acid used in titrating to the first end-point. Determine the total bases by a separate titration and calculate the primary amine by difference.

Separation and determination of ammonia—Neutralise the sample, which may contain 15 to 20 mg.-equivalents of amine, in an ice-bath with hydrochloric acid, dilute to about 25 ml. with water, and add 25 ml. of sodium cobaltinitrite solution. Allow to stand for 1·5 hr. in the ice-bath, add 40 ml. of Methyl Cellosolve, and leave in the ice-bath for 30 min. more. Use suction to filter off the precipitate of sodium diammonium cobaltinitrite on an asbestos filter, wash with 50 ml. of 50 per cent. Methyl Cellosolve, and transfer the precipitate, with the asbestos, to a distilling flask. Add an excess of sodium hydroxide solution, distil the liberated ammonia into boric acid solution, and titrate in the usual manner. If the presence of both ammonia and water has prevented titration of secondary amines in the original sample, they may now be determined by the general procedure, after recovery from the filtrate from the cobaltinitrite precipitate by the following method. Transfer the filtrate, immediately after filtration, to a distilling flask, add an excess of sodium hydroxide solution, distil 50 ml. into 45 ml. of cold methanol, dilute to 100 ml., and take a 10- to 15-ml. aliquot for the azomethine method.

Results on 17 known mixtures of amines (not including ammonia) show errors varying between − 1·6 and + 1·3 per cent. of the quantity of secondary amine present. Allylamines, methallylamines, ethanolamines, diethylenetriamine, dimethylamine plus butylamine, and piperidine plus ethylamine give end-points that are satisfactory, but less sharp than others. Diethanolamine reacts with salicylaldehyde, probably forming an oxazolidine, but this compound hydrolyses during the titration if 5 ml. of water are added. The secondary amino group of diethylenetriamine is only weakly basic, but its basicity is greatly increased by the addition of salicylaldehyde. The probable reason for this is discussed.

The use of benzaldehyde for azomethine formation leads to unsatisfactory results for a number of secondary amines. A reason for this is suggested and several reasons for the adoption of salicylaldehyde are stated.

The ammonia separation is not applicable in presence of methylamine since the latter yields a cobaltinitrite that is only slightly soluble. Recovery of higher-boiling amines is not complete by the prescribed procedure; a loss of about 5·6 per cent. is shown for *n*-butylamine. W. C. JOHNSON

Determination of Halogens in Organic Substances by Hydrogenation. M. Pesez and P. Poirier (*Bull. Soc. Chim.*, 1948, 379–381M)—In the Raney nickel reduction method of Schwenck *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 576) the alloy must be added very slowly, at 0° C., to avoid the excessively vigorous evolution of hydrogen. The authors' method provides a simple means of avoiding this difficulty, but it is not applicable to certain volatile compounds such as chloro- and bromo-benzenes in which the halogen is not sufficiently mobile.

Procedure—In a test tube dissolve a quantity of the substance equivalent to 10 to 15 ml. of 0·1 *N* or 0·02 *N* silver nitrate, in 10 ml. of methanol. Add 10 ml. of 20 per cent. sodium hydroxide solution, 2 g. of zinc powder, and 0·5 g. of coarsely powdered Raney alloy containing 33 per cent. of nickel. Fit a reflux condenser and heat over boiling water, maintaining a constant evolution of hydrogen for 1 hr. Allow to cool, decant the liquid, wash the residue of zinc by decantation with two 20-ml. portions of water. Add 10 ml. of nitric acid to the mixed liquid and washings and complete the determination by the Volhard titration.

The effect of the Raney nickel is apparently catalytic since the reduction is incomplete in its absence. The following compounds yield satisfactory results: *p*-chloro-*m*-cresol, *p*-chloronitrobenzene, tribromophenol, bromocamphor, bromotriphenylethylene, bromosuccinimide, eosin, 1-(2′-bromoethyl)-6-methoxynaphthalene, dibromotyrosine, 2 : 4-dibromoandrosterone, di-iodotyrosine, and di-iodothymol. W. C. JOHNSON

Determination of Glycerophosphoric Acid. Application to Phosphatides. T. Cahn, J. Houget, and R. Agid (*Bull. Soc. Chim.*, 1948, 666–668M)—The addition of barium acetate solution to a dilute aqueous solution of sodium glycerophosphate produces no precipitate, but the further addition of 5 volumes of methyl alcohol precipitates the barium salt almost quantitatively. The precipitate is less soluble on heating. After boiling, filtering, washing with 83 per cent. methyl alcohol, and drying over sulphuric acid in a vacuum desiccator the material weighs 110 to 130 per cent. of the theoretical value. After reprecipitation with methyl alcohol from an acetate solution the substance is not pure barium glycerophosphate; it contains only about 9·2 per cent. of phosphorus (theory = 9·82 per cent.), but entrainment of the solutes compensates for losses due to solubility and the results are correct to within 2 per cent. when calculated according to the theoretical factor 1 g. of barium glycerophosphate = 0·542 g. of glycerophosphoric acid. The experiments were carried out with pure specimens of sodium α- and β-glycerophosphate.

Procedure—Dissolve in 7 ml. of water a quantity of sodium glycerophosphate equivalent to 0·05 to 0·20 g. of glycerophosphoric acid. Add 0·7 ml. of a 50 per cent. solution of barium acetate (equivalent to 0·217 g. of glycerophosphoric acid) and 40 ml. of methyl alcohol, boil for a moment on a water-bath, and allow to stand 2 hr. Centrifuge, using 5 ml. and 3 ml. of 83 per cent. methyl alcohol to wash the precipitate into the tube. Dry the precipitate and dissolve it in the centrifuge tube, using 4 ml. and three 1-ml. quantities of 0·01 *N* acetic acid containing 0·1 *M* sodium acetate, centrifuging after each treatment so as to decant only clear liquid. Add to the solution 0·36 ml. of 0·25 *N* barium hydroxide (the *p*H should now be about 9·3) and, immediately, 40 ml. of methyl alcohol. Complete as after the initial precipitation, and weigh.

Determination of glycerophosphoric acid in phosphatides—Saponify the material by boiling with 1·0 *N* alcoholic potassium hydroxide for 2 hr. Add water and sulphuric acid until the mixture is 50 per cent. with respect to alcohol and 0·1 *N* with respect to alkali, and extract the unsaponifiable matter with light petroleum. Make acid with sulphuric acid and extract the fatty acids. Make just alkaline to phenolphthalein with potassium hydroxide and evaporate to dryness on a water-bath. Redissolve in 5 ml. of water, add 50 ml. of methyl alcohol, filter, and wash the residue of potassium sulphate with 90 per cent. methyl alcohol. Evaporate the alcoholic solution to dryness and redissolve in 7 ml. of water. From this point follow the process of precipitation with barium acetate described above. The barium salt obtained contains only 5·7 to 8·5 per cent. of phosphorus, varying with the nature of the phosphatide. It is impossible, therefore, to complete the analysis simply by weighing the precipitate. Dissolve the first precipitate in 7 ml. of acetic acid - acetate solution and determine the total phosphorus and the inorganic phosphorus, and thence the glycerophosphate phosphorus by difference.

The precision claimed is to within 3 to 4 per cent. Boiling with 1·0 *N* alcoholic potassium hydroxide for 2 or 7·5 hr. causes no appreciable hydrolysis of glycerophosphoric acid. W. C. JOHNSON

Chromatographic Analysis of Synthetic Mixtures of Isomeric Trimethylglucoses. R. A. Boissonnas (*Helv. Chim. Acta*, 1947, **30**, 1689–1703)—The methylglucoses are hydrogenated and converted into the corresponding *p*-phenylazobenzoyl ("azoyl") glucitols, which are separated chromatographically on alumina. Azoyl methylglucitols corresponding to the following sugars were separated from binary mixtures: 2 : 3 : 4 : 6-tetramethylglucose, 2 : 3 : 4-, 2 : 3 : 6-, 2 : 4 : 6- and 3 : 4 : 6-trimethylglucose, and 2 : 3-dimethylglucose.

Procedure—Neutralise alumina (Merck) by stirring for 3 hr. on a water-bath with 3 *M* nitric acid and washing to neutrality with hot water. Dry at 120° C. for 6 hr. and heat to dull red-heat for 1 hr. To obtain alumina of activity I (characterised by the method of Brockmann and Schodder, *Ber.*, 1941, **74**, 73) add 5 per cent. by weight of distilled water to the calcined alumina, shake for 10 hr., and dry at 120° C. for 6 hr. For alumina of activity III add 3 per cent. by weight of distilled water to alumina I and shake for 10 hr. Spent alumina is recoverable by washing with boiling methanol containing 2 per cent. of water, and re-processing as above.

Use 1 ml. of alumina per mg. of substance to be treated chromatographically, and make the height of the alumina column seven times its diameter. To separate the tetramethyl from the trimethyl compounds, use benzene as solvent and developer, and alumina III as adsorbent. To separate the trimethyl compounds from each other and from the dimethyl compound, use chloroform as solvent and developer, and alumina I as adsorbent.

At the bottom of the tube place a cotton wool plug covered with a circle of filter paper. Pour a suspension of floridin XXF in the solvent into the tube to form a column 2 to 5 cm. long after sedimentation, tapping the tube during this process. Pass through two column lengths of solvent not letting the floridin become dry. In one operation pour in a suspension of alumina in the solvent, tapping the tube during sedimentation. Cover the top of the adsorbent with a filter-paper disc and pass a column length of solvent, taking care not to dry out the column. The solvent flows under gravity; its linear velocity should be 2·5 to 4 mm. per min., and is controlled by the height of the bed of floridin.

Pass the solution (1 to 2 per cent.) of the azoyl methylglucitols through the column to form an initial band 3 to 5 mm. deep. Develop by passing solvent (20 to 30 column lengths) until the lower band reaches the bottom of the alumina column. Using benzene and alumina III, the 1 : 5-diazoyl-2 : 3 : 4 : 6-tetramethylglucitol remains at the top of the column in a sharp, intense band, while the trimethyl compound forms a diffuse band below, Using chloroform and alumina III, the upper band. at times diffuse, is in the middle of the column, while the lower band, sharp and intense, is at the bottom. When development is complete, pass two column lengths of light petroleum (b.p. 45° to 60° C.) and let the column dry for 15 min. Remove the different bands from the tube with a spatula and put each into a small tube plugged at the bottom with cotton wool. Elute the bands with acetone; elution is immediate and, with two washings, quantitative. Evaporate the eluate to dryness, and estimate the residue, dissolved in benzene, photometrically; crystallise from 98 per cent. ethanol and identify by a melting-point.

G. H. TWIGG

Polarographic Estimation of the Total Amount of Fat in Soaps and Soap Powders. S. Fiala and V. Jancik (*Coll. Czech. Chem. Comm.*, 1948, **13**, 30–36)—The method is based on the principle that a soap solution will readily precipitate the cations of light and heavy metals by an exchange reaction with the alkali metal cations of the soap. If the concentration of these ions is measured polarographically before and after adding the soap, the fat content of the soap can be calculated. Cadmium ions have been shown to be the most convenient for this purpose.

Procedure for solid soaps—Dissolve 10 g. of the soap in 100 ml. of 80 per cent. ethyl alcohol and neutralise the alkali present with 0·5 *N* hydrochloric acid, using phenolphthalein as indicator. After boiling the solution, neutralise the alkali liberated, and then dilute the solution accurately to 1 litre. Pipette 20 ml. of this solution into a 200-ml. volumetric flask containing 20 ml. of 0·25 *N* cadmium sulphate. After mixing the contents, add 20 ml. of saturated potassium chloride solution, dilute to 200 ml. with distilled water, and filter the solution. Examine a sample of the solution polarographically, after removing dissolved oxygen, and measure the height of the cadmium step produced. The percentage of fat in the soap is then given by

$$m\,(H - h) \times 10^6/198{\cdot}85\,H\,w\,\mathrm{N}$$

where h is the height of the step given by this solution, H is the height of the step given by an exactly similar solution to which no soap has been added, m is the weight in milligrams of cadmium in 20 ml. of standard solution, w is the weight of soap in grams dissolved in 1 litre, and N is the neutralisation number of the fatty acids in the soap.

The cadmium sulphate stock solution should be stored free from carbon dioxide, and standardised gravimetrically as cadmium pyrophosphate.

To determine the neutralisation number of the fatty acids in the soap, shake 300 ml. of the soap solution with 10 ml. of 5·0 *N* hydrochloric acid and 30 ml. of ether. Wash the ether layer with water until the washings are neutral and filter the extract through anhydrous sodium sulphate into a dry, weighed flask. After removing the ether by evaporation, cool and weigh the flask and its contents. Dissolve the fat in 50 ml. of ethyl alcohol and titrate the solution with 0·5 *N* hydrochloric acid.

Procedure for soap powders—Dissolve 5 g. of the soap powder in 30 ml. of absolute alcohol, dilute the solution to 1 litre with alcohol, and filter through a filter paper. Pipette 20 ml. of the filtrate into a 200-ml. volumetric flask, and neutralise the alkali with 0·5 *N* hydrochloric acid. Add 100 ml. of water and 20 ml. of 0·25 *N* cadmium sulphate, followed by 20 ml. of saturated potassium chloride, and dilute to 200 ml. The subsequent procedure is the same as that described for solid soaps.

The results obtained by the above procedures are in good agreement with those given by other methods, and the method effects a considerable saving of time and materials. J. G. WALLER

Estimation of Residual Wool Fat in Tops Oiled with B.M. (Blended Mineral) Oil. E. W. Clark and W. L. Thomas (*J. Text. Inst.*, 1948, **39**, T255–T259)—The method is based on the difference in the mean refractive indices of B.M. oil and wool fat, *i.e.*, 1·4707 and 1·4809, respectively, at 40° C.

Procedure—Extract 10 to 15 g. (accurately weighed) of the top with light petroleum (b.p. 40° to 60° C.), weigh the extract, warm it until it is thoroughly molten, and transfer some to the prism of an Abbé refractometer (maintained at 40° ± 0·1° C.). The wool-fat content is obtained from a graph or table (both reproduced in the original paper) relating the refractive index and percentage composition of the extract, and based on the refractive indices of known mixtures of B.M. oil and wool fat. [A condensed form of the table is given below.] The method is unaffected by the normal variations obtained in different batches of B.M. oil, or in wool fats from different wools, or by variations in temperature within the above limits. It can be adapted to oils other than B.M. oil by constructing an appropriate reference table or graph as described above. The method can be combined with the usual analytical process of solvent extraction, as 0·05 g. of extract suffices for a refractometer reading. The method is not applicable to tops combed in oil which have been stored for some time. This difficulty might be overcome by saponifying the extract, and determining the refractive index of the unsaponifiable matter, but a new reference table based on the appropriate standard mixtures would then have to be compiled.

Refractive indices at 40° C.	Percentages of wool fat in mixtures of B.M. oil and wool fat
1·4707	nil
1·4710	3·5
1·4720	14·8
1·4730	25·5
1·4740	36·0
1·4750	46·0
1·4760	56·0
1·4770	65·6
1·4780	75·0
1·4790	83·9
1·4800	92·5
1·4809	100·0

J. GRANT

Statistical Study of the Variables of Alpha-Cellulose Methods. A. S. O'Brien, L. K. Reitz, and R. C. Bloom (*Paper Trade J.*, 1948, **127**, Aug., *T.A.P.P.I. Sect.*, 51–54)—The analysis of variance method applied to the determination of the α-cellulose contents of chemical cotton, high-alpha sulphite pulp, semi- and un-bleached krafts, and bleached sulphite wood pulps, led to the following conclusions. The correction for the moisture content of the sample must be determined with great care; preferably, the necessity to do so should be eliminated by the use of vacuum-dried pulp (dried at 60° C.). The method of rinsing with acetic acid, the size of the sample (1 or 3 g.), the amount of stirring during mercerisation, and the addition of the caustic soda in portions or all at once, have little or no influence on the results. The use of ground samples gives lower results than when thin sheets are used, and the use of alcohol and ether to hasten drying is not trustworthy. Dilution of the 17·5 per cent. caustic soda solution to 8·3 per cent., followed by an additional 1 hr. of standing before filtering (Amer. Chem. Soc. method), produced results slightly lower than those obtained by diluting to 8·3 per cent. with stirring, and then filtering at once (standard T.A.P.P.I. method

T203 m–44); either method gave results much lower than those obtained by filtering directly after treatment with the 17·5 per cent. caustic soda solution, without dilution. J. Grant

Determination of Nicotine in Nicotine Sulphate and in Tobacco Leaves. G. Métayer (*Chimie Analyt.*, 1948, **30**, 109–113)—Existing methods, which are reviewed, are time-consuming and require costly apparatus; they need the skill of qualified operators and some involve the use of inflammable solvents. The method now described is said to be free from these disadvantages.

Distillation apparatus—A 250-ml. Claisen flask with a Vigreux column in the side-arm is fitted with a rubber stopper bearing a 50-ml. tap funnel and an air inlet tube drawn off to a capillary at its lower end and fitted at the top with a rubber tube and screw-clip for regulation. This flask stands in an oil-bath. The receiver is a distillation flask closed with a stopper through which the side-tube of the Claisen flask passes to the centre of the receiver. The receiver is connected to a water-pump.

Procedure—Weigh 0·3 to 0·4 g. of nicotine sulphate or a quantity of tobacco dependent upon the approximate nicotine content, as follows: for tobacco containing 4 per cent. or more of nicotine, 1 g.; 2·7 to 4 per cent., 3 g.; less than 2·5 per cent., 5 g. Transfer the sample to the Claisen flask and add a quantity of potassium hydroxide pellets equal to the weight of nicotine sulphate or 2 per cent. of the weight of the tobacco sample. Add 110 ml. of ethylene glycol, reduce the pressure in the apparatus to 12 to 15 mm. and heat for 10 min. with the oil-bath at 120° to 140° C. to eliminate ammonia and readily volatile amines. Then raise the oil-bath temperature to 180° to 200° C. and distil 200 ml. of glycol at a steady rate while adding a further 120 ml. from the top-funnel. Efficient cooling is necessary. Transfer the contents of the receiver to a beaker, wash the receiver with water, add the washings to the distillate and dilute to 300 ml. with water. Titrate the nicotine with 0·05 N sulphuric acid to the neutral grey colour of an indicator prepared as follows. Dissolve 0·1 g. of methyl red in 7·4 ml. of sodium hydroxide solution [presumably 0·05 N], add 25 ml. of a 0·5 per cent. aqueous solution of methylene blue, and dilute to 500 ml.

The quantity of potassium hydroxide is specified as a result of experiments which showed that if the proportion was more or less than 2 per cent. of the weight of the (undried) sample of tobacco the results by the volumetric procedure were higher than those obtained by precipitating and weighing the nicotine as the dipicrate. [It is not indicated how the very small quantities of solid potassium hydroxide are to be weighed.]

The method gives results in good agreement with those obtained by the method of Schloesing (*Cours de Chimie analytique*, Paris, 1913). Only one type of tobacco, Nijkerk, was found not to be amenable to the procedure, but this variety gives difficulties in other methods. W. C. Johnson

Analytical Method for the Examination of Technical Polyvinyl Products. W. J. Taat and W. van der Heul (*Chem. Weekblad.*, 1948, **44**, 393–397)—The usual methods for the examination of polyvinyl products take several days, and are unable to distinguish a co-polymer from a mixture of polyvinyl acetate and chloride. The new method is based on the solvent power of *o*-dichlorobenzene, and it is essential that this solvent shall be free from non-volatile compounds. This is ensured by using the fraction of the commercial material boiling between 155° and 183° C.

Procedure—Cut the sample, freed from fabric, into pieces, and dissolve 0·5 g. in 15 ml. of *o*-dichlorobenzene with the aid of gentle heat. Add to the solution 30 ml. of butanone, and centrifuge. Pour off the liquid, wash the residue twice with a mixture of *o*-dichlorobenzene and butanone, and dry it at 105° C. to constant weight. This gives the weight of filler or pigment. Evaporate the solution and wash liquors on the water-bath to 30 ml. and add 100 ml. of warm, 96 per cent. alcohol. After the mixture has been kept for 1 hr. on the water-bath, filter it on a glass filter 1H3, and wash the residue with 96 per cent. alcohol. Dry the residue at 100° C., and weigh it. This gives the weight of polyvinyl chloride and/or chloride - acetate copolymer. An estimation of chlorine is carried out on this mixture to determine the proportions of the two components. Evaporate the filtrate from the last coagulation on the water-bath to constant weight; redissolve it in 80 ml. of acetone and add 40 ml. of water. Filter off the coagulum, wash with 50 per cent. acetone, and dry. This is the polyvinyl acetate. Evaporate the filtrate and dry at 80° C. to give the weight of plasticiser. G. Middleton

Inorganic

Rapid Estimation of Free Sulphur. L. Peyron (*Bull. Soc. Chim.*, 1948, 482–483M)—The general principle of the method is said to be capable of extension to the estimation of other substances. It consists in dissolving the substance in a liquid and mixing the solution with another liquid in which the substance is insoluble, so that a colloidal dispersion is obtained. The opacity or refractive index of the dispersion is then determined as a measure of the concentration of the substance.

Procedure—Heat the sample, containing about 5 mg. of sulphur, with 5 ml. of pure pyridine at 60° C. for 1 hr. with occasional shaking. Filter and add 1 ml. of the filtrate, dropwise with shaking, to 10 ml. of water. Determine the opacity with a suitable optical or photo-electric instrument and interpret the results from a calibration curve based on known amounts of sulphur treated in the same manner. Reference dispersions of sulphur must be renewed after 2 to 3 hr. Bituminous samples must first be treated with a solvent that has no action upon the sulphur. W. C. Johnson

Semi-micro Determination of Halogens and Sulphur [and Mercury] in Substances containing Mercury. M. Jurecek (*Coll. Czech. Chem.*

Comm., 1947, 12, 455–466)—Sodium carbonate at a low red heat is a satisfactory absorbent for chlorine, bromine, and sulphur when the mercury compound is heated in a current of oxygen. Iodine is not completely absorbed by sodium carbonate and for compounds containing this element silver dispersed in magnesium oxide must be used. The mercury vapour passes on and can be determined by absorption on gold wool, but if nitrogen is also present, mercury must be determined separately by combustion with oxidising agents in an indifferent gas.

Apparatus—(*a*) Supremax glass combustion tube, length 45 cm., internal diameter 10 mm., wall thickness 0·7 to 1 mm., with neck. When mercury is to be determined, a transparent fused quartz tube, of the same dimensions but with a ground-on absorption tube in place of the neck, is used. The straight part of the absorption tube is 5 cm. long, 5 mm. in diameter, and filled with gold wool. (*b*) Platinum contact star, length 5 cm. (*c*) Glazed porcelain boat, 6 cm. long and occupying two-thirds of the section of the tube. (*d*) Hard glass "ampoule," with loop, 9 mm. diameter, 5 cm. long, which is placed near the mouth of the tube to prevent the substance from subliming back. (*e*) Three sheet-iron heating mantles, 10, 6, and 4 cm. long, respectively. (*f*) Mariotte bottle, 3-litre capacity. (*g*) Bubble counter, calibrated by means of (*f*).

Special reagents—(*a*) Silver dispersed in magnesium oxide: mix magnesium oxide to a paste with a solution of an equal weight of silver nitrate, evaporate the water on a water-bath, heat on an asbestos mat until no more brown vapours are evolved, and finally ignite at red heat. (*b*) Mercuric nitrate solution, 0·04 *N*: dissolve 4·4 g. of mercuric oxide in the smallest quantity of diluted nitric acid (1 + 1) and dilute with water to 1 litre. Determine the factor of this solution against 3- to 8-ml. quantities of 0·04 *N* sodium chloride diluted to 40 ml., acidified with 2 drops of nitric acid and containing 0·01 g. of sodium nitroprusside. Titrate with the mercuric nitrate solution to the production of an opalescence that lasts 1 min. To determine the factor on 0·04 *N* potassium bromide proceed similarly, but dilute to 60 ml. (*c*) Sodium hypochlorite solution: introduce 7 g. of chlorine into 100 ml. of 12 per cent. sodium hydroxide solution.

Determination of chlorine, bromine, and sulphur—Insert the porcelain boat, filled with anhydrous sodium carbonate, into the combustion tube to a distance of 10 cm. from the neck. Boil the platinum star with diluted nitric acid (1 + 1), ignite it gently and insert it in the tube 1 cm. from the boat. At a further interval of 5 to 8 cm. place a platinum boat containing 20 to 30 mg. of the substance for analysis and then, at a distance of 4 cm., the "ampoule." Close the tube temporarily with a plug of cotton wool, arrange the 10-cm. and 6-cm. heating mantles, in contact, to cover respectively the porcelain boat and the star and thus to extend about 3·5 cm. beyond the boat. Adjust the 4-cm. mantle between the platinum boat and the "ampoule." Replace the cotton wool plug with a rubber stopper through which passes the capillary of the bubble counter, which is connected in turn with an oxygen holder or cylinder with a fine regulator valve. Arrange a shield over the part of the tube containing the porcelain boat and the star, to conserve the heat. Regulate the oxygen at 7 to 9 ml. per min. heat the mantle over the star to a bright red with a double Bunsen burner, and that over the boat of sodium carbonate to a dull red with a 7-cm. burner, then light a Bunsen burner under the third mantle and ignite the substance in the usual manner, advancing the burner and the mantle toward the star. The combustion should occupy 45 min. The mercury forms droplets on the cold tube beyond the porcelain boat; any white crystalline sublimate indicates that the combustion has been too rapid. Having advanced the short mantle to touch the central one, extinguish the burners and allow the apparatus to cool with the oxygen still passing. Transfer the contents of the porcelain boat to a 100-ml. flask, add water and 1 ml. of concentrated nitric acid and dilute to 40 ml. for a chloride titration or 60 ml. for a bromide titration. Using sodium nitroprusside as indicator titrate with 0·04 *N* mercuric nitrate to the production of an opalescence lasting 1 min. Bromine can be titrated separately as follows. Dissolve the sodium carbonate from a second combustion in 20 ml. of water in a 250-ml. Erlenmeyer flask with a ground-glass stopper, neutralise to methyl red with *N* hydrochloric acid, and make alkaline with 1 drop of *N* sodium hydroxide. Add 5 ml. of sodium hypochlorite solution and 2 g. of sodium chloride, and pass a current of carbon dioxide for 15 min. Heat over boiling water for 10 min., add 5 ml. of 10 per cent. sodium formate solution and some glass balls, and boil for 15 min. Cool, acidify with 25 ml. of 2 *N* sulphuric acid, add 1 to 2 drops of 5 per cent. ammonium molybdate solution and 1 to 2 g. of potassium iodide, stopper, and allow to stand for 5 min. Titrate the liberated iodine with 0·1 *N* sodium thiosulphate and starch as indicator. Determine sulphur as barium sulphate by the usual procedure.

Determination of iodine—Follow the above procedure but fill the porcelain boat with silver dispersed in magnesium oxide. After the combustion, dissolve the absorbent in 5 ml. of diluted nitric acid (1 part of acid, sp.gr. 1·4 + 2 parts of water). Dilute with 25 ml. of water, boil for 10 min., allow to cool, collect the silver iodide in a 12G4 filter tube, dry in a Pregl copper block, and weigh.

Simultaneous determination of halogens, sulphur, and mercury in substances containing no nitrogen—Follow the procedure for chlorine and bromine or iodine but use the quartz tube with the weighed absorption tube in place. The gold is kept cool by a piece of cloth, which is moistened from time to time. Having completed the combustion, extinguish the movable burner and that under the platinum star and increase the oxygen current to 12 to 15 ml. per minute. Move back the 10-cm. mantle by 3 cm. and place the full flame of a micro-burner 3 cm. behind the deposit of mercury. Extinguish the burner under the porcelain boat and advance the micro-burner at 3 mm. per minute to within 1 cm. of the gold wool. Repeat once or twice the heating of the tube between the boat and the gold, and allow to cool in the current of oxygen. Detach the absorption tube and attach to its ground end a calcium chloride tube, attach the other end to the Mariotte bottle and draw 300 to 500 ml. of air through the tube at about 15 ml. per minute. After weighing, remove the mercury, in preparation for the next determination, by careful ignition and cooling while maintaining a current of dry air.

W. C. JOHNSON

Separation and Determination of Fluorine. **E. Rinck** (*Bull. Soc. Chim.*, 1948, 305–324M)—A comprehensive and critical review of published methods, some of which are also re-investigated by the author. [This abstract deals only with the author's contributions and with the methods he favours.]

Gravimetric methods—Precipitation as calcium fluoride by a method quoted from *Handbuch für das Eisenhuttenlaboratorium*, 1939, **1**, 17, 134, 153, gives results from 0·44 to 0·70 per cent. low. The method is considered satisfactory, although owing something to a compensation of errors. Precipitation as lead chlorofluoride with suitable precautions (Fischer and Peisker, *Z. anal. Chem.*, 1933, **95**, 225) is accurate to within 0·3 per cent., but the volumetric development (*infra*) of this method is preferred.

Volumetric methods—The direct titration of chloride in precipitated lead chlorofluoride offers no advantage over the gravimetric method, but the following indirect method is recommended as accurate to within 0·5 per cent.

The fluoride solution must contain less than 100 mg. of fluorine in not more than 200 ml. and must be free from chloride. Neutralise the solution to methyl orange and add a known volume of 0·1 N sodium or potassium chloride. Add, drop by drop, a slight excess of 0·1 *N* lead nitrate to the hot solution and set aside for 12 hr. Check for neutrality, transfer the solution and the precipitate to a standard 250- or 500-ml. flask and dilute to the mark. Filter through a dry paper, rejecting the first portions and, to an aliquot of not more than 40 ml., add 10 ml. of a freshly prepared solution of diphenylamine blue. Titrate with 0·1 *N* silver nitrate, with vigorous agitation, until a permanent violet tint is obtained. The indicator is prepared by adding 0·1 ml. of a solution of 1 g. of diphenylamine in 100 ml. of sulphuric acid and 1 ml. of 0·1 *N* potassium dichromate to 10 ml. of 5 *N* sulphuric acid.

Titration with neutral *M*/16 aluminium chloride of fluoride or silicofluoride solutions saturated with sodium chloride, in presence of methyl red as indicator (Kurtenacker and Jurenka, *Z. anal. Chem.*, 1930, **82**, 210), is strongly recommended. In absence of interfering ions the error is less than 0·5 per cent. Sulphate, in concentration equal to that of the fluorine ion, causes the results to be 3 per cent. high. Perchlorate gives a 1 per cent. error at the same concentration. Carbonate causes large errors; for this reason the aluminium chloride solution must be protected with a soda-lime tube and the evaporation of alkaline fluoride solutions must be avoided.

Titration with thorium nitrate in the presence of sodium alizarin sulphonate (Willard and Winter, *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7) is the best method. The present author's experiments give rise to the following observations and recommendations: (*a*) The most satisfactory end-points are obtained when 1 mg., or less, of fluoride is titrated; then the thorium fluoride remains in colloidal solution. With larger quantities, the rose-coloured lake that marks the end-point is adsorbed on the precipitated fluoride. (*b*) 0·01 Mg. of fluoride in 30 ml. of solution can be determined to within 0·001 mg. (*c*) The maximum volume of solution recommended is 50 ml., preferably 25 to 30 ml., for quantities down to 0·01 mg. The colour is highly dependent on the indicator concentration; the volume of the solution should not, therefore, increase by more than 10 per cent. during the titration. (*d*) Thorium nitrate solution: maximum concentration, 0·05 *N*; recommended, 0·01 *N* (= 0·0025 *M*), used in a micro-burette graduated in 0·01 ml. (*e*) Indicator solution: 1 g. of sodium alizarin sulphonate in 1 litre of water. Add 1 drop to 10 ml. of solution. (*f*) The end-point is best observed in diffuse daylight. It should be the same colour as that obtained in standardising the thorium nitrate, but a comparison solution thus prepared rapidly becomes deeper in colour. The cobalt nitrate - sodium dichromate standard tint of Matuszak and Brown (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 100) does not provide an identical colour, especially when variable concentrations of fluoride and of other ions modify the colour of the end-point, but the standard proves useful with practice. (*g*) Aqueous solution is better than the 50 per cent. alcoholic solution used by Willard and Winter (*loc. cit.*) except for about 0·1 mg. of fluoride, which is more precisely titrated in dilute alcohol. The presence of alcohol does not affect the proportionality between the quantity of fluoride and the volume of thorium nitrate solution required (*cf.* Dahle *et al.*, *J. Assoc. Off. Agric. Chem.*, 1938, **21**, 459, 468). (*h*) The *p*H at the end-point should be 3·6 to 3·9 for alcoholic solutions and this may be achieved by using a chloroacetate buffer (Hoskins and Ferris, *Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 6), although the latter must be freshly prepared, otherwise hydrolysis products diminish the *p*H. Contrary to the findings of the original authors, the F : Th relationship is constant under these conditions. In aqueous solutions the correct *p*H of 3·25 to 3·45 at the end-point is attained by adjusting the original solution to a pale rose colour with hydrochloric acid or sodium hydroxide and then adding 1 drop of 2 to 3 per cent. acetic acid. An excessive concentration of acetate ions renders the end-point less sharp. The use of a solution of thorium nitrate in 1·2 *N* acetic acid (Matuszak and Brown, *loc. cit.*) leads to a lack of proportionality between fluoride and thorium. (*i*) The quantity of thorium nitrate solution required to form the lake in a blank experiment must be deducted from each titration.

Colorimetric methods—The zirconium nitrate - sodium alizarin sulphonate method of De Boer and Basart (*Z. anorg. Chem.*, 1926, **152**, 203) is the most useful.

Separation of fluoride from interfering substances by distillation—The author recommends the method of Willard and Winter (*loc. cit.*), but uses a separate steam generator. Sulphuric acid at 155° to 160° C. or perchloric acid at 140° to 145° C. may be employed, but the latter is preferable since any sulphuric acid that may be entrained has a severe influence on certain methods of determination, notably the thorium nitrate titration (Hoskins and Ferris, *loc. cit.*). A mixture of equal volumes of sulphuric acid and 72 per cent. perchloric acid may be employed for minerals more easily decomposed by sulphuric acid. The use of phosphoric acid is deprecated, since it is readily entrained and has a strong influence on the subsequent titration. If phosphate is present in the substance for analysis the distillation should be repeated, the second time at a temperature not higher than 135° C. The rate of distillation is without influence, apart from the risk of entrainment, if too rapid. Quantities of fluorine of the order of 1 mg. may be recovered completely in 100 ml. of distillate in 15 to 20 min., even from insoluble fluorides. Gelatinous silica retards the distillation of the hydrofluosilicic acid. If the SiO_2 : F ratio is 20 : 1, 300 ml. of distillate must be taken; if the ratio is 50 : 1, 400 ml. of distillate are necessary. A greater ratio necessitates a preliminary separation. Ferric and ferrous ions also retard the distillation. Aluminium should be separated prior to the distillation when it is present in more than ten times the fluorine concentration. The suitability of the reagents and of the glassware must be checked by a blank distillation and titration. Pyrex glass has been found to lead unaccountably to high results.

Evaporation of solutions of alkali fluorides—Reynolds and Hill (*Ind. Eng. Chem.*, *Anal. Ed.*, 1939, **11**, 21) and McClure (*Ibid.*, 1939, **11**, 171) found that losses occurred when solutions of alkali fluorides were evaporated. This is confirmed, but the reason is not discovered. Evaporation should be avoided, or if this is not possible the *p*H should be kept at 6 to 8 and a platinum or Jena glass vessel should be employed. Absorption of carbon dioxide even by a neutral solution rules out subsequent titration with aluminium chloride.

W. C. Johnson

Colorimetric Method for Determining Fluoride Ions in Presence of Phosphates. D. Monnier, R. Vaucher, and P. Wenger (*Helv. Chim. Acta*, 1948, **31**, 929–932)—When the bleaching effect of fluoride ions on pertitanic acid is employed for the colorimetric estimation of fluoride, the *p*H and the concentration of peroxide must be strictly controlled.

Solutions—Place 26·7 ml. of an 18 per cent. solution of titanous chloride in a litre measuring flask, dilute with a small volume of boiled water, add 100 ml. of concentrated sulphuric acid and 60 ml. of perhydrol, and fill to the mark with boiled distilled water. This solution may be kept for several months. For use, dilute it ten-fold. Prepare a standard fluoride solution containing 1·947 g. of ammonium fluoride in 1 litre and dilute this solution 100-fold to obtain a solution containing 0·01 mg. of fluoride per ml.

Procedure—Measure 7·5 ml. of 1·2 *N* hydrochloric acid, 3 ml. of the reagent solution, and 1 ml. of perhydrol into a 50-ml. flask, and dilute to the mark. Measure the optical density of this solution by means of the Hilger Spekker absorptiometer with a 4-cm. cell, filters H503 and Ilford 601, and the tungsten filament lamp. Repeat the procedure, adding increasing quantities of the diluted fluoride solution before the final dilution, and construct a calibration curve.

The curve follows the Beer - Lambert law for quantities of fluoride from 30 to 160 μg. per 50 ml. of solution. The method is not very sensitive, the molecular extinction coefficient being 163. The maximum error is about 3 μg. of fluoride.

Estimation of fluorine in teeth—In order to preserve the correct conditions it is necessary to vary the quantity of acid according to the type and quantity of the sample, as follows.

Finely powdered substance	Quantity in g.	1·2 *N* HCl in ml.
Enamel	0·3	12
"	0·1	9
"	0·05	8·5
"	0·01	8
Dentine	0·3	10·5
"	0·1	8·5
"	0·05	8
"	0·01	8
Entire tooth	0·3	11·5
" "	0·1	9
" "	0·05	8·5
" "	0·01	8

Mix the prescribed quantity of acid with 3 ml. of the reagent solution, pour the mixture on to the sample, wash the mixing vessel with 10 ml. of water, and add this to the reaction mixture. Allow the reaction to proceed for not more than 10 min. with occasional stirring. Transfer to a 50-ml. measuring flask, add 1 ml. of perhydrol to compensate for any reduction due to organic matter, and make up to the mark. Filter through two dry papers, check the complete clarity of the filtrate, measure its light absorption, and read the fluorine content from the calibration graph. W. C. Johnson

Determination of Silver with Ascorbic Acid. E. C. Stathis (*Anal. Chem.*, 1948, **20**, 271)—*Procedure*—Weigh a sample containing not more than 0·1 g. of silver, and treat it with 5 ml. of 6 *N* nitric acid; then evaporate the solution almost to dryness on the steam-bath. Dilute to 20 ml. with distilled water and heat the solution to between 90° and 100° C. before adding slowly 10 ml. of freshly prepared, 2 per cent. ascorbic acid solution. Maintain at this temperature for 15 min., then filter the precipitate of metallic silver on to a porcelain filter crucible, wash it with hot water, and ignite it.

Results—On 10 samples containing from 11 to 138 mg. of silver the maximum error was 0·2 mg. Lead, copper, bismuth, cadmium, nickel, and zinc do not interfere. M. E. DALZIEL

New Conductometric Method for the Titration of Univalent Thallium using Potassium Selenocyanate. R. Ripan and E. Popper

(*Gazz. Chim. Ital.*, 1942, **72**, 439–445)—Thallium acetate can be titrated conductometrically, potassium selenocyanate being used as precipitant.

Procedure—The titre of the solution of selenocyanate was determined potentiometrically with silver nitrate and volumetrically using fluorescein as indicator. The solution of thallium acetate was titrated conductometrically with potassium chromate and with potassium iodide for comparison with the results with the selenocyanate. The thallium salt, which should be neutral or slightly alkaline, is placed in the titration cell and the reagent added slowly with continual stirring. Thallium selenocyanate separates immediately as a crystalline precipitate that settles rapidly leaving the solution perfectly clear. Equilibrium is immediately established after each addition so that the reading can be made at once, giving the advantage of rapidity in working. During the precipitation the conductivity changes very little, but when quantitative precipitation has been attained the conductivity increases rapidly and the two branches of the curve intersect at an angle sufficiently acute to permit great precision. For the most accurate results the concentration of the thallium acetate should be about 0·025 M and that of the selenocyanate about 0·5 M.

The results obtained are in close agreement with those found with potassium chromate or potassium iodide.

The inverse titration, using the thallium salt as reagent, is less precise, the angle between the two branches of the curve being much greater.

A. H. BENNETT

Critical Study of the Gravimetric Determination of Calcium in Presence of Magnesium. S. Peltier and C. Duval

(*Analyt. Chim. Acta*, 1947, **1**, 408–417)—Published procedures for the gravimetric determination of calcium have been investigated to establish which methods are suitable for use in presence of large quantities of magnesium. The methods studied included precipitation as oxalate in various ways, precipitation as tungstate, molybdate, or sulphate (from alcoholic solution), and also the use of 8-hydroxyquinoline for removal of magnesium before determining the calcium. The precipitations were carried out with chloride solutions containing 10 to 40 mg. of calcium and, at least, 20 times that weight of magnesium.

The use of 8-hydroxyquinoline for preliminary separation of the magnesium gave low results owing to entrainment of calcium with the oxine precipitate. The procedures that gave satisfactory results with a single precipitation were (i) precipitation of calcium as tungstate, (ii) precipitation as oxalate in the cold in presence of glycerol, (iii) precipitation as oxalate at pH 3·7 in presence of formic acid, and (iv) precipitation as sulphate from 60 per cent. alcohol solution (re-precipitation as oxalate is recommended). Precipitation as molybdate is unsatisfactory (*cf.* Iwin and Grinstein, *Z. anal. Chem.*, 1944, **127**, 20). The tungstate procedure is recommended for its speed and ease of execution.

Determination of calcium in presence of magnesium (*tungstate method*)—Adjust the solution, of volume about 70 ml. and containing about 0·035 g. of calcium ion, to pH 7 to 8 and heat to 80° C. Add 2 ml. of 19 per cent. sodium tungstate solution and stir, without touching the walls of the containing vessel, until precipitation is complete. Allow to cool and, after 30 min., filter the precipitate on a weighed, No. 3 sintered-glass crucible, washing with 20 ml. of hot water. Dry for 1 hr. at 110° C., cool, and weigh.

The precipitation should be carried out in a solution containing only calcium, magnesium, and alkali metals. Large amounts of ammonium chloride prevent the precipitation. H. J. CLULEY

Determination and Separation of Calcium and Magnesium. A. Lassieur

(*Chimie Analyt.*, 1947, **29**, 197–201)—Methods for determining calcium and magnesium in presence of one another and in presence of lithium are described.

DETERMINATION OF CALCIUM IN PRESENCE OF MAGNESIUM—Using bromophenol blue as indicator, adjust to pH 4 to 4·5 a solution containing not more than 1·5 g. of calcium oxide and 0·5 g. of magnesium oxide per litre and occupying a volume of about 300 ml. Heat to boiling and add 2 g. of solid ammonium oxalate; continue to boil for 2 min. Filter immediately and wash the paper and precipitate with warm water. Ignite and weigh as calcium oxide.

Serious contamination of the calcium oxalate by magnesium occurs if the precipitate is allowed to stand in the cold before filtration. A satisfactory separation can be made in presence of alkali salts without the need for re-precipitation.

DETERMINATION OF MAGNESIUM—*Lithium absent*—To the filtrate from the calcium oxalate precipitate add an excess of ammonium dihydrogen phosphate, followed by 5 ml. of aqueous ammonia in excess. Stir to promote the crystallisation of the magnesium ammonium phosphate. If precipitation occurs almost immediately, allow to stand for 4 hr. before filtering, otherwise set aside for 16 hr. Collect the precipitate on a suitable filter, wash with diluted aqueous ammonia (1 in 10) and ignite at a red heat and weigh.

Addition of 5 g. of ammonium chloride is without effect.

Lithium present—Lithium is co-precipitated with magnesium on adding ammonium phosphate, as is shown by the flocculent nature of the precipitate, and must be separated as follows. Dissolve the precipitate in hydrochloric acid, add ferric chloride equal to 20 times the weight of phosphates present, and precipitate the iron with aqueous ammonia, at the boiling-point. Filter, evaporate the filtrate to dryness, and ignite. Extract the residue with acetone, which dissolves the alkali chlorides present, leaving the partially decomposed magnesium chloride undissolved. Filter, re-dissolve the residue in acid and precipitate the magnesium with ammonium phosphate by the procedure already described. P. M. C. Proffitt

New Volumetric Method of Determining Mercuric Oxide with Alkali Thiocyanate. F. Burriel and F. Pino (*Informac. Quim. Analit.*, 1947, **1**, 46–49, 68)—The determination is based on the reaction

$$4KCNS + HgO + H_2O \rightarrow K_2[Hg(CNS)_4] + 2KOH$$

and is a modification of the Büllmann - Thanlow method (*Bull. Soc. Chim.*, 1921, (4), **29**, 587) in which potassium iodide is used.

Procedure—Treat 0·1 to 0·2 g. of the sample in an Erlenmeyer flask with 15 g. of potassium thiocyanate and enough water to give a volume of 100 ml. Keep the flask stoppered to prevent absorption of carbon dioxide, and if necessary warm to effect dissolution. Cool, and titrate the liberated potassium hydroxide with 0·1 *N* sulphuric acid, using as indicator methyl orange or the screened methyl red - methylene blue indicator; 1 ml. of 0·1 *N* sulphuric acid is equivalent to 0·01083 g. of mercuric oxide. A. F. Sturgess

Cerimetric Determination of Copper and Antimony. R. Přibil and T. Chlebovsky (*Coll. Czech. Chem. Comm.*, 1947, 12, 485–501)—The copper, in 5 to 6 *N* hydrochloric acid solution, is reduced to the univalent state by means of a solution of chromous chloride. The slight excess of chromous chloride is removed and the copper is titrated with a standard solution of ceric sulphate. The reactions are conducted in a closed titration vessel in an atmosphere of carbon dioxide and the end-points are detected potentiometrically, a platinum spiral as indicator electrode and a calomel reference electrode being used. Nickel, cobalt, manganese, zinc, lead, antimony, and arsenic do not interfere, but iron is titrated with the copper. In a solution containing 40 ml. of concentrated hydrochloric acid and 20 ml. of concentrated sulphuric acid in each 100 ml. both copper and antimony can be determined. The solution is reduced as above and the copper is titrated; iodine chloride solution is then added to act as a catalyst in the oxidation of the antimony by the titrant.

The original paper should be consulted for discussion of the experimental development of the method and for further details of the determination of copper in alloys, and the determination of copper, antimony, and arsenic in alloys with lead and tin. L. A. Dauncey

Systematic Analysis of Cations by the Polarographic Method. II. Conditions for Determining Elements of the Arsenic Sub-Group. M. A. Portnov and V. P. Povelkina (*J. Anal. Chem. Russ.*, 1948, **3**, 85–91)—*Tin*—With barium chloride or sodium chloride as supporting electrolyte, concentrations of stannous tin from 0·0008 to 0·02 *N* in *N* hydrochloric acid, obtained by dissolution of tin in 6 to 8 *N* hydrochloric acid, followed by dilution and passage of hydrogen gas for 1 hr., gave wave-heights proportional to concentration. In diluted sulphuric acid or in 10 per cent. sodium hydroxide solution, proportionality occurred only with tin concentrations not less than 0·003 *N* (reduction potential, about −0·4 v.), but in the alkaline solution indications, not further investigated, of two unstable waves were obtained. In a mixture of equal volumes of 5 *N* sulphuric acid and ethyl alcohol, with sodium or potassium sulphate as supporting electrolyte and in an atmosphere of hydrogen, wave-heights were strictly proportional to tin concentration over the range 0·0004 to 0·0054 *N*. With stannic tin in hydrochloric acid solution, the wave-height slowly decreased with time.

Antimony—Conditions given by Suchy (*Chem. News*, 1931, **143**, 213) and Cozzi (*Annali Chim. Appl.*, 1934, 442, *cf.* also Lingane, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 583) for determining antimony in hydrochloric acid medium were found satisfactory. In 10 *N* sulphuric acid, antimony is reduced at −0·2 v., and wave-heights were proportional to concentration. In oxalic acid medium, tervalent antimony is reduced at −0·4 v., and proportionality was also found. In 10 to 20 per cent. sodium hydroxide solution, the reduction potential is −0·2 v., but proportionality occurred only at concentrations of 0·006 *N* and above. In neutral solution, the reduction potential is −0·6 v. In a 1 : 1 mixture of 5 *N* sulphuric acid and ethyl alcohol the reduction potential is −0·27 v., and proportionality between wave-height and concentration occurred over the range 0·00036 to 0·0058 *N*, but with concentrations below 0·0007 *N* two waves tended to form. For the determination of antimony in this medium the total wave given by a solution containing gelatin and sodium or potassium sulphate as supporting electrolyte, and freed from oxygen by means of hydrogen, should be measured.

Arsenic—In alkaline medium, arsenic is not reduced at the dropping mercury electrode. In sulphuric acid solution three waves appeared, the first two only could be used for analytical work since the third is unstable. In alcoholic sulphuric acid solution (see above), three waves with half-wave potentials, −0·64, −0·88, and −1·15 v. appeared. With the first of these waves, concentration and wave-height were proportional over the range of arsenic concentration 0·00030 to 0·0025 *N*.

Determination of tin, antimony, and arsenic when present together—In 0·5 *N* sulphuric acid well defined waves of all three elements, with two of the arsenic waves missing, appeared, but the wave-heights of antimony and tin were not proportional to their concentrations. In more concentrated sulphuric acid solutions the reduction potential of the "stable" arsenic wave became more positive and the wave coalesced with that of tin. In alcoholic sulphuric acid solution (see above) in presence of gelatin, results for each of the three elements present together were satisfactory provided the polarograms were taken without delay after the preparation of the solution. Furthermore, lead, which accompanies the arsenic group elements in certain methods of analysis, did not interfere, and there was no need to filter off the lead sulphate before the electrolysis.

G. S. SMITH

Determination of Alumina in Silica Bricks by Precipitation with 8-hydroxyquinoline T. R. Lynam and A. Nicholson (*Trans. Brit. Ceramic Soc.*, 1948, **47**, 116–119)—Cashmore and Cowling (*Ibid.*, 1946, **45**, 309) proposed for determining alumina in clays and firebricks a rapid method in which the alumina is precipitated with 8-hydroxyquinoline from a buffered aqueous extract of a sodium hydroxide fusion of the sample. This method cannot be applied to the determination of alumina in silica bricks, the precipitation of the aluminium oxinate being partly or wholly suppressed. Determinations on synthetic silica-alumina mixtures showed that the silica-alumina ratio is a critical factor, incomplete precipitation of the alumina occurring with ratios greater than 20 : 1. For the analysis of silica bricks and other highly siliceous materials, the preliminary removal of silica with hydrofluoric acid is recommended.

Procedure—To 1 g. of the finely-ground sample add 5 drops of sulphuric acid and 8 ml. of hydrofluoric acid, evaporate to dryness, and ignite. Fuse the residue with 6 to 8 g. of sodium carbonate, extract the cold melt with boiling water, filter, and wash with hot water. To the filtrate add hydrochloric acid dropwise until the solution is acid to methyl orange, boil for 2 min., and cool. To eliminate interference from any iron present, add 1 ml. of 50 per cent. hydroxylamine hydrochloride solution and 20 drops of 1 per cent. *o*-phenanthroline in diluted hydrochloric acid (1 + 1) solution. Add 15 ml. of 2·5 per cent. 8-hydroxyquinoline in 5 per cent. acetic acid solution, heat to 70° C., add 30 per cent. ammonium acetate solution until a permanent precipitate results and then add 10 ml. more. Allow to stand for 10 min. and filter through a pulp pad, washing with water. Redissolve the precipitate through the pad with 75 ml. of nearly boiling, diluted hydrochloric acid (1 + 1), and wash with hot water. Transfer the solution to a flask provided with a stopper, dilute to 200 ml., and cool.

Add standard bromate-bromide solution (1 ml. $\equiv$ 1 mg. Al_2O_3) from a burette, slowly and with constant shaking. When 5 ml. short of expected requirements have been added, add 3 drops of 1 per cent. indigo carmine solution and continue titrating until the green colour disappears. Add 3 more drops of indigo carmine solution, titrate to a golden-yellow colour, and add 3 ml. more of bromate-bromide solution. Stopper the flask, shake well, and allow to stand for 5 min. Add 10 ml. of 20 per cent. potassium iodide solution and titrate back with standard sodium thiosulphate solution, using starch solution as indicator. H. J. CLULEY

Volumetric Determination of Tervalent Iron Based on the Use of Tartrates. A. V. Pavlinova (*J. Anal. Chem. Russ.*, 1948, **3**, 7–10)—A solution of ferric chloride, neutralised to methyl orange, yields on treatment with sodium potassium tartrate solution, also neutralised to methyl orange, an amount of acid, as measured by titration to the phenolphthalein end-point, that is proportional to the iron content of the solution, provided hydrolysis is prevented by adding calcium chloride. Tests showed that 0·0189 g. of iron combine with tartaric acid equivalent to 0·0656 g. of sodium potassium tartrate. Thus the complex contains the iron and the tartrate residue in the molecular proportions of 4 : 3. Titration of the acid liberated showed that three equivalents are formed for each equivalent of iron. The amount of calcium chloride necessary to yield theoretical results was the equivalent of not less than 0·4 ml. of 40 per cent. (half-saturated) calcium chloride solution for each 20 ml. of solution. Titration of the calcium chloride solution alone showed that 1 ml. required 0·06 ml. of 0·1 *N* potassium hydroxide for neutralisation to phenolphthalein, but tests with various amounts of the solution added to a ferric tartrate solution showed that a correction based on this figure was faulty. The results were identical when the final concentration of calcium chloride was between the limits of 0·5 and 2 per cent.

Procedure—To 20 ml. of ferric chloride solution, neutral to methyl orange and containing 0·1890 to 0·05670 g. of iron [this was the range in the experiments], add 2 or 3 ml. of 10 per cent. sodium potassium tartrate solution, and 2 drops of phenolphthalein indicator solution, and titrate with 0·1 *N* potassium hydroxide, free from carbonate, until the solution, after becoming nearly colourless, turns pink. Then add 0·5 to 1 ml. of 40 per cent. calcium chloride solution and titrate the now decolorised solution to the final end-point at the rate of 1 or 2 drops per 15 sec.

The maximum errors were +0·42 and −0·35, and the average error ±0·20 parts in 100.

A similar method has been used by the author for determining aluminium. G. S. SMITH

Determination of Oxidic Inclusions in Steel. H. Henkel (*Z. anal. Chem.*, 1947, **128**, 26–41)—Klinger and Koch (*Arch. Eisenhuttenw.*, 1937–38, **11**, 571) have described a procedure for the determination of oxidic inclusions in steel, in which a preliminary separation of the inclusions is effected by an electrolytic method. Experience of this procedure has led the author to suggest a number of modifications, including a preliminary heat treatment to reduce to a minimum the amount of carbide present in the sample. This facilitates the separation of the inclusions and minimises the danger of iron carbide remaining unchanged during the subsequent treatment of the inclusions and thus giving rise to high values for ferrous oxide. A further modification is the use of a gravimetric method for determining silica, which is claimed to give more trustworthy results than the absorptiometric silicomolybdate procedure recommended by Klinger and Koch.

Apparatus—The electrolytic separation of the inclusions is carried out in the apparatus described by Klinger and Koch (*loc. cit.*). The anodic compartment is fitted with a filter and a circulating system for the anolyte. The catholyte is contained in a porous pot treated with agar-agar, which acts as a diaphragm. The anolyte contains 150 g. of sodium citrate, 18 g. of potassium bromide, and 2 g. of potassium iodide per litre, and the catholyte is 10 per cent. cupric bromide solution. The sample, which is made the anode, is rotated mechanically during the electrolysis.

Preparation of the samples—Two samples, of weight 60 to 80 g. and length 8 to 10 cm., are required for each complete determination. Stamp the samples for identification and carry out a heat treatment suitable for decomposition of the carbides, quenching in brine. (The procedure for the heat treatment will depend on the composition of the sample.) Remove the oxide layer by filing, clean with a steel brush, and weigh the sample.

Separation of the inclusions—Carry out the electrolysis using a maximum current of 0·8 amp. and a rate of flow of anolyte of about 100 drops per minute. Check the *p*H of the anolyte periodically; values in the range 6·8 to 7·0 should be obtained. Stop the electrolysis after passing 20 ampere-hr. of current, the duration of electrolysis being normally about 30 hr. Remove the residue of inclusions, which will be partly on the filter of the anodic compartment and partly adhering to the undecomposed sample, and wash well with water. Dry and reweigh the undecomposed sample to determine the weight of sample corresponding to the inclusions obtained.

By this procedure obtain inclusion residues from two separate samples and treat one residue by procedure (A) and the other by procedure (B).

(A) *Determination of alumina, ferrous oxide, and combined manganous oxide*—Add 0·45 ml. of diluted hydrochloric acid (2 + 3) to 50 ml. of cupric bromide solution (200 g. in 300 ml. of water). Transfer the inclusion residue and 30 ml. of the acidified cupric bromide solution to a vessel fitted with a steam jacket. Pass into the solution oxygen-free nitrogen, begin the steam heating, and continue for 5 min. after the steam passes through the jacket uncondensed. Remove the solution and repeat the procedure for 2 min. with a further 15 ml. of acidified cupric bromide solution. Filter off the residue, washing with water, then with 10 per cent. sodium carbonate solution, and finally with water.

Ignite the residue in platinum and fuse with 6 g. of sodium carbonate. Extract the cake with water, add 1 drop of alcohol, boil and filter, washing with water. Retain the residue for the determination of ferrous oxide and combined manganous oxide.

Carefully neutralise the filtrate with diluted hydrochloric acid (2 + 3), warm to remove carbon dioxide, and dilute to 100 ml. Prepare a blank solution by fusing 6 g. of sodium carbonate, dissolving in water and neutralising with diluted hydrochloric acid (2 + 3). To compensate for any colour due to chromate in the test solution, measure the absorption of the test solution in a 5-cm. cell at 430 mμ., with the mercury lamp, using water as a reference solution. Add the corresponding quantity of potassium chromate to the blank solution and dilute to 100 ml.

For the aluminium determination take 10 ml. of the test solution, dilute to about 30 ml., and make just alkaline to phenolphthalein with 5 per cent. sodium hydroxide solution. Carefully neutralise with 1·37 *N* hydrochloric acid (53 ml. of hydrochloric acid diluted with 450 ml. of water), adding 10 drops in excess. Add 10 ml. of 0·15 per cent. erio-chromcyanin solution (prepared 3 to 12 hr. previously) and 20 ml. of acetate buffer solution (274 g. of ammonium acetate, 109 g. of sodium acetate, and 6 ml. of acetic acid per litre), and dilute to 100 ml. After 15 min., measure the absorption of the solution in a 0·5-cm. cell at 530 mμ. with the tungsten lamp, using as a reference solution 10 ml. of the blank solution similarly treated. Calculate the alumina content of the sample from a calibration curve prepared, using standard aluminium solutions.

Dissolve the residue obtained after water extraction of the sodium carbonate melt in 15 ml. of hot, diluted sulphuric acid (1 + 3) and a few drops of hydrogen peroxide, add 10 ml. of diluted nitric acid (1 + 4), and boil. Dilute, add 10 ml. of 0·85 per cent. silver nitrate solution and 10 ml. of 50 per cent. ammonium persulphate solution and heat to 60° C. After oxidation, cool and dilute to 100 ml. Measure the absorption of the solution in a 2-cm. cell at 530 mμ. with the tungsten lamp, using water as a reference solution. The manganese figure, calculated from a calibration curve prepared, using standard manganese solutions, represents the percentage of chemically combined manganous oxide in the sample.

For the ferrous oxide determination take as much as possible of the solution used for the manganese determination, add an excess of aqueous ammonia, and boil. Filter, wash with hot water, and dissolve the precipitate in 10 ml. of diluted hydrochloric acid (1 : 1). Add a few drops of hydrogen peroxide and evaporate to 2 ml. Dilute to about 80 ml., add 1 ml. of 40 per cent. potassium thiocyanate solution, and dilute to 100 ml. Measure the absorption of the solution in a 1-cm. cell at 470 mμ. with the tungsten lamp, using water as a reference solution. Calculate the ferrous oxide content of the sample from a calibration curve prepared by means of standard iron solutions.

(B) *Determination of silica and free manganous oxide*—Extract the inclusion residue with 30 ml. of glycine solution (5 g. of glycine in 30 ml. of water), filter, and evaporate the filtrate, which contains the free manganous oxide, to dryness. Ignite, fuse with potassium hydrogen sulphate, and extract with diluted sulphuric acid (1 + 2). Determine the manganese by the procedure as in (A). The manganese figure obtained represents the free manganous oxide in the sample and must be added to the amount found by procedure (A) (combined manganous oxide) to give the total manganous oxide in the sample.

To determine silica, wash the residue from the glycine extraction with water and digest with 20 ml. of hot, diluted hydrochloric acid (1 + 10) to decompose sulphides. Filter, wash with water and then with 10 per cent. sodium carbonate solution until the washings give a negative test for silica with ammonium molybdate solution and dilute hydrochloric acid. (The washing with sodium carbonate solution removes freshly precipitated silica formed by the action of hydrochloric acid on silicides present in the inclusions; silica present as such in the original sample remains undissolved.)

Ignite the residue and fuse with sodium carbonate. Transfer the cake to a platinum dish, decompose with 20 ml. of diluted hydrochloric acid (2 + 3), and evaporate to dryness. Add 50 ml. of concentrated hydrochloric acid, boil down to 20 ml., add 10 ml. of gelatin solution (0·5 g. in 100 ml. of concentrated hydrochloric acid), and allow to stand for 30 min. Dilute and filter, washing with hot water containing dilute hydrochloric acid and finally with hot water alone. Ignite the silica precipitate and weigh on a micro-balance.

H. J. Cluley

Determination of Selenium in Steel. N. A. Tananaev and V. I. Murasheva (*J. Anal. Chem. Russ.*, 1948, **3**, 3–6)—Selenium can be determined in the insoluble residue left after dissolution of steel in sulphuric acid, as only negligible traces of volatile compounds of selenium were found in the evolved gases obtained by treatment of 30-g. portions of steel with 150 ml. of diluted sulphuric acid (1 + 4).

Procedure—Dissolve 20 g. of steel in a 500-ml. conical flask by gentle heating on a sand-bath with 150 ml. of diluted sulphuric acid (1 + 4). Filter the solution through a filtering crucible without suction, wash the insoluble matter with hot water several times, then wash back the residue into the original flask, pour 50 ml. of concentrated nitric acid through the crucible, again wash the crucible with hot water, collecting the acid and washings in the flask containing the residue, and boil the solution until oxides of nitrogen are no longer evolved. Transfer the solution to a porcelain dish and evaporate it to dryness over boiling water. Dissolve the dry residue in concentrated hydrochloric acid, filter through a filtering crucible, rinsing the dish three times with hydrochloric acid, and wash the insoluble matter in the crucible with hydrochloric acid twice, and then twice with hot water. Treat the filtrate at 40° to 50° C. with 50 ml. of hydrochloric acid saturated in the cold with hydrogen sulphide, and allow to stand for 24 hr. Then filter through a filtering crucible, washing successively with hydrochloric acid, hot water, alcohol, and ether. Dry at 35° to 40° C. and finally at 105° to 110° C. to constant weight. The residue is selenium.

Alternatively, the determination can be finished volumetrically. Dissolve the selenium in concentrated nitric acid, make alkaline with aqueous ammonia solution and then acid with acetic acid, dilute to 400 ml. with water, add 3 g. of urea and 3 g. of potassium iodide, and titrate the liberated iodine after 1 to 3 min. with thiosulphate in presence of starch.

Tellurium does not interfere, as it remains in solution during the hydrogen sulphide treatment in the very acid medium used.

Steels containing from 0·03 to 0·095 per cent. of selenium were analysed by this method and by the A.S.T.M. method. Results agreed to 0·002 per cent. (of the sample). Selenium-free steels were also dissolved and known amounts of selenious acid were added to the solutions. Recovery of selenium was satisfactory, *e.g.*, amounts added 0·0025 g., 0·0085 g., 0·0636 g. of selenium—amounts found 0·0028 g., 0·0083 g., and 0·0632 g., respectively.

The method is considered to be as accurate as, and much simpler than, the A.S.T.M. method.

G. S. Smith

Preparation of Standard Chromous Sulphate or Chromous Chloride Solutions of Determinate Concentration. J. J. Lingane and R. L. Pecsok (*Anal. Chem.*, 1948, **20**, 425–428)—Because the chromous ion is the most powerful reductant used as a standard solution, its preparation and storage are difficult. A solution of exactly determinate strength is prepared by reduction of a chromic salt of known purity by amalgamated zinc, the same vessel serving for the reaction and for storage.

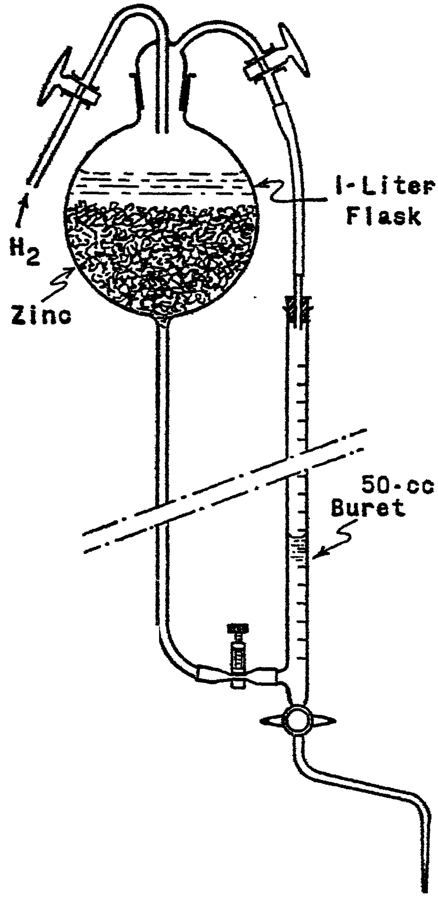
Method—In the apparatus used the 1-litre, round-bottomed flask is one-half to two-thirds full of pure zinc amalgam. The solution is kept under pure hydrogen generated in a Kipp apparatus and passed through a U-tube containing some chromous sulphate in 0·1 *N* sulphuric acid in contact with amalgamated zinc to remove oxygen. The burette is easily removed for cleaning and is clamped in position, the reservoir being supported on a rubber-covered ring. Amalgamate about 1 kg. of mossy zinc (arsenic-, lead-, and iron-free) by stirring for a few minutes with a mercuric chloride solution in dilute hydrochloric acid, and then wash it with cold water. Between 600 and 700 ml. of chromium solution can then be accommodated in the flask.

To prepare 0·1000 *N* chromous sulphate in 0·1 *N* sulphuric acid, dissolve 29·431 g. of pure, dried potassium dichromate in about 500 ml. of water in a 2-litre Erlenmeyer flask and add 27·8 ml. of concentrated sulphuric acid. Through a long-stemmed funnel in the neck of the flask add slowly 75 ml. of pure 30 per cent. hydrogen peroxide solution, and heat the solution near the boiling-point until all the oxygen is evolved (about 20 min.). Cool to room temperature and dilute the solution to 2 litres in a volumetric flask. Rinse the storage flask twice with 100-ml. portions of the solution, and transfer the remainder. Close the flask and connect the hydrogen generator. Only a small amount of the gas is necessary to protect the solution from air and to replace the solution withdrawn.

If a solution that is normal with respect to sulphuric acid is required, add 50 ml. more concentrated sulphuric acid before diluting to 2 litres. Reduction of a full reservoir of solution takes a few hours if the flask is shaken occasionally, but complete disappearance of the chromic green colour is easily recognised. The solution in 0·1 *N* acid is stable for about 1 week, and that in *N* acid, for about 3 weeks.

For solutions of chromous chloride, appropriate amounts of hydrochloric acid replace the sulphuric acid.

Potentiometric titration is necessary because of the green colour of chromic ion, but the end-point is sharp. A suitable titration apparatus consists of a three-necked balloon flask. An inert gas, freed from oxygen as the hydrogen was, is passed through a tube in a rubber stopper in the central neck, and a platinum-wire indicator electrode is wound round the tube. One side-neck carries a salt bridge, filled usually with 1 to 2 *N* sulphuric acid, with ends closed by internal ground-glass plugs (Irving and Smith, *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 480) for connection to a saturated calomel reference electrode through a saturated potassium chloride solution. The burette tip passes through a hole in a stopper in the third neck, slightly over-large, to allow the escape of gas. The gas stream is started 15 to 20 min. before the titration and maintained during it. When precipitates are produced, mechanical stirring is advantageous.



Standardisation against copper sulphate dissolved in diluted hydrochloric acid (1 + 1) gives results agreeing with the determinate normality to within ±0·1 per cent. Direct titration against dichromate is erratic, but addition of a slight excess of ferrous ammonium sulphate (free from ferric ion) and titration of the resultant ferric ions gives an excellent curve, agreement again being to within ±0·1 per cent.

M. E. Dalziel

Physico-chemical Analysis of Systems Important in Analytical Chemistry. XIII. The System $CoSO_4$ - $K_4Fe(CN)_6$ - H_2O. I. V. Tananaev and M. I. Levina (*J. Anal. Chem. Russ.*, 1948, **3**, 31–40)—Measurements of solubility and light absorption in the system $CoSO_4$ - $K_4Fe(CN)_6$ - H_2O have shown that the interaction between cobalt ions and potassium ferrocyanide takes place in three stages, with the formation of two double salts, $5Co_2Fe(CN)_6,K_4Fe(CN)_6$ and $4Co_2Fe(CN)_6,3K_4Fe(CN)_6$, and solid solutions of variable composition. X-Ray spectra proved the individuality of the two double salts. From the phase diagram a method of determining cobalt was indicated.

Procedure—To 23·84 ml. of a solution of cobalt sulphate, containing 0·1320 g. of cobalt, placed in a 250-ml. graduated flask, was added from a burette 0·08320 *M* potassium ferrocyanide in quantity sufficient to coagulate the colloidal solution that formed first. The solution was then made up to the mark and shaken, and a portion of the clear liquid removed and titrated with 0·1 *N* potassium permanganate to give the excess of ferrocyanide over that required to form

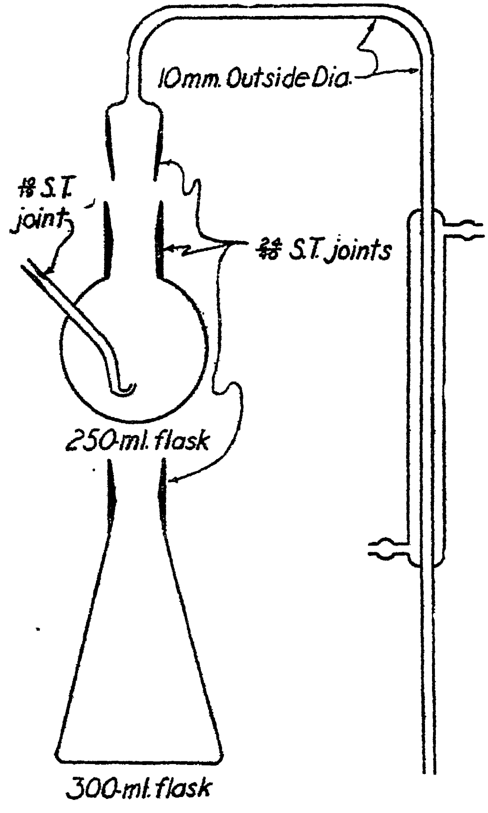
$$4Co_2Fe(CN)_6,3K_4Fe(CN)_6.$$

The amount of ferrocyanide solution that has to be added to cause coagulation is about double the theoretical. No adverse effect is produced by adding larger excesses of ferrocyanide.

G. S. Smith

Analysis of Thorium - Chromium Mixtures. R. E. Ewing and C. V. Banks (*Anal. Chem.*, 1948, **20**, 233–234)—Since thorium is unaffected by most oxidising agents the chromium can be oxidised and determined titrimetrically, the most satisfactory oxidising agent being perchloric acid. Any chromyl chloride evolved is condensed and titrated with the bulk solution. Thorium can be precipitated as oxalate in presence of chromium if sufficient oxalic acid is present to form the $Cr(C_2O_4)_3'''$ complex also, but slight contamination of the precipitate with chromium occurs. Accuracy is increased but speed forfeited if the chromium is first volatilised as chromyl chloride. The apparatus is shown.

Procedure—Determination of thorium—Treat a sample containing between 200 and 300 mg. of thorium in a beaker with 10 to 15 ml. of concentrated nitric acid and 5 to 10 drops of diluted hydrofluosilicic acid (1 ml. of 48 per cent. acid + 50 ml. of water). Heat until dissolution is complete and transfer to the round-bottomed flask with 3 drops of 30 per cent. hydrogen peroxide solution and 35 ml. of 60 per cent. perchloric acid solution. Place a 300-ml. Erlenmeyer flask containing 150 ml. of water beneath the condenser so that its end is just submerged. Bubble nitrogen through the solution at a rate of 1 or 2 bubbles per second and heat the flask. When oxidation has begun replace the nitrogen stream with hydrogen chloride, generated by dropping concentrated hydrochloric acid into concentrated sulphuric acid. When nearly all the chromium has been removed, transfer the solution to a beaker and concentrate it to 5 ml. to remove the excess of perchloric acid, and dilute to 300 ml. Heat to the boiling-point, and add 5 ml. of filter pulp and 5 g. of oxalic acid. Digest hot for 15 min., cool, then filter, wash, and ignite the precipitate and weigh it as ThO_2.



Determination of chromium—Treat a sample containing between 70 and 75 mg. of chromium in a beaker with 10 to 15 ml. of concentrated nitric acid and 5 to 10 drops of hydrofluosilicic acid. When dissolution is complete, transfer to the 300-ml. Erlenmeyer flask and add 3 drops of 30 per cent. hydrogen peroxide solution and 20 to 25 ml. of 60 per cent. perchloric acid solution. The condenser dips into 150 ml. of water contained in a 200-ml. Erlenmeyer flask. Heat until oxidation is complete and cool the flask. Return the distillate to the flask and boil the solution for 20 min. to expel chlorine. Cool, and add an excess of 0·1 *N* ferrous sulphate (in 1 per cent. sulphuric acid), and titrate back with 0·1 *N* tetrasulphatoceric acid, using 1 or 2 drops of ferroin as indicator (14·8662 g. of 1 : 10-phenanthroline monohydrate and 6·9505 g. of heptahydrated ferrous sulphate in 1 litre of solution).

Results—In samples containing between 180 and 319 mg. of thorium in presence of 80 to 522 mg. of chromium, thorium can be determined to within 0·2 mg. In samples containing between 68·5 and 75·6 mg. of chromium in presence of 42 to 210 mg. of thorium, chromium can be determined to within 0·2 mg. M. E. Dalziel

Thorium in Monazite Sand. Separation and Determination by Precipitation from Homogeneous Solution. H. H. Willard and L. Gordon (*Anal. Chem.*, 1948, **20**, 165–169)—The main precipitation reactions employed are of the type in which the precipitant is produced uniformly throughout the sample solution by the decomposition of a suitable compound, instead of being added as a relatively localised stream of solution. The method described consists of three main parts: decomposition of monazite sand with perchloric acid, precipitation of thorium and rare earths, and separation of thorium from the rare earths. The advantages claimed over previous methods are greater speed and accuracy. Critical discussion and experimental investigations leading to the following method are given in some detail.

Decomposition of monazite sand—Weigh 50 g. into a lipped, wide-mouth, 1000-ml. conical flask, and boil vigorously with 200 ml. of perchloric acid (70 per cent.) for 1 to 1·25 hr. Do not cover the flask, as the return of water reduces the effectiveness of the acid. Cool somewhat, add 50 ml. more of perchloric acid and then, slowly and with agitation of the liquid, 300 ml. of water. Add, slowly and with vigorous shaking, 18 g. of hydrazine dihydrochloride crystals; this substance reduces and makes soluble the precipitated basic cerium phosphate. Warm gently for 1 hr. to remove gaseous decomposition products. The solid residue consists of zircon, quartz, rutile, ilmenite, and dehydrated silica. Cool the flask and add 3 g. of purified diatomaceous earth (Filter-cel). Mix thoroughly and filter through a medium grade 15-cm. paper supported by a small platinum cone. Wash the residue with diluted perchloric acid (1 + 3). Remove the paper and residue to a small beaker and macerate with a few ml. of diluted perchloric acid (1 + 3), and decant through an 11-cm. paper. Wash the residue several times, and then dilute the combined filtrates to 1 litre with diluted perchloric acid (1 + 3).

Precipitation of thorium and rare earth oxalates—To 25 ml. of the perchloric acid solution in a 600-ml. beaker add silica-free concentrated aqueous ammonia solution drop by drop, mixing thoroughly, until a permanent turbidity is produced. Add 10 ml. of concentrated hydrochloric acid and shake occasionally for 5 min. Add 100 ml. of water and 6 g. of methyl oxalate. Use slow mechanical stirring and heat the solution at 70° to 85° C. for 30 min. The oxalate dissolves, the solution becoming clear, and then hydrolyses, and precipitation of oxalates occurs. Add a hot solution of 8 g. of oxalic acid in 280 ml. of water and stir for a further 30 min., keeping the solution warm. Cool, and filter through an 11-cm., ashless paper of medium porosity, and wash the precipitate ten times by decantation with a 2 per cent. solution of oxalic acid containing 40 ml. of concentrated hydrochloric acid per litre. Put the filter paper in the beaker containing the precipitate, add 20 ml. of concentrated nitric acid and 5 ml. of perchloric acid (70 per cent.), and warm gently until fumes of perchloric acid appear. Cool, add 200 ml. of water and 6 g. of methyl oxalate, heat and stir for 30 min. as above, add a hot solution of 8 g. of oxalic acid in 200 ml. of water, and heat and stir for 30 min. more. Cool, filter, and wash five times by decantation. Decompose the paper and oxalates by heating with 20 ml. of nitric acid and 10 ml. of perchloric acid until fumes of perchloric acid appear. The solution is then quite free from phosphate.

Separation of thorium—Cool the solution of perchlorates, and add 1 g. of potassium iodide in 25 ml. of water and then a filtered solution of 8 g. of urea, 10 g. of ammonium chloride, and 3 ml. of formic acid (90 per cent.) in 150 ml. of water. Dilute to 350 ml. and, using silica-free aqueous ammonia solution, adjust the *p*H to 4·45 ± 0·02 with the aid of a calibrated glass electrode. Stand a stirring rod, with a 1-mm. indentation in the lower end, in the beaker and heat so that, after bringing the solution to boiling-point, a steady stream of bubbles rises from the indentation. Boil for 95 to 97 min. after the appearance of opalescence. The final *p*H should be 5·4 to 6·2. If it is less than 5·4 more boiling is necessary to precipitate all the thorium. If it is above 6·2 some rare earths may precipitate, but will be removed in the second precipitation. Filter and wash ten times with a hot solution, adjusted to *p*H 5·6, containing 20 g. of ammonium nitrate and 8 ml. of formic acid per litre. Remove most of the thorium precipitate on the beaker walls with a "policeman." Dissolve the precipitate on the paper in hot 2 *N* hydrochloric acid and collect the solution in the precipitation beaker. Wash the paper thoroughly. Add 1 g. of potassium iodide and carry out the precipitation as described above. The final *p*H after 95 to 97 min. boiling should be 5·4 to 6·0. Filter and wash the precipitate ten times. Remove most of the precipitate adhering to the beaker with a "policeman" and add to the filter. Put 10 ml. of diluted hydrochloric acid (1 + 1) into the beaker, insert the stirring rod, cover, and boil very gently for 10 min. Rinse the cover glass and beaker walls, add aqueous ammonia solution drop by drop until bromocresol purple becomes purple and warm for 1 to 2 min. Filter the few milligrams of gelatinous precipitate on the filter containing the basic thorium formate and wash five times. Ignite in platinum for 2 hr. at 950° C., remove silica with hydrofluoric and sulphuric acids, and ignite for 2 hr. at 950° C. or to constant weight. L. A. DAUNCEY

Thermal Behaviour of Analytical Precipitates. T. Duval and C. Duval (*Analyt. Chim. Acta*, 1948, **2**, 97–102, 103–104, 105–109, 110–114)—*Sodium*—The precipitated chloride loses moisture below 407° C. and is then stable to 878° C. The perchlorate is precipitated as the monohydrate, but is anhydrous at 130° C., and the anhydrous salt does not decompose below 471° C.; conversion to chloride is complete at about 620° C. The sulphate loses moisture from ordinary temperatures and becomes anhydrous between 90° and 878° C. The antimonate is precipitated with varying amounts of water, dehydration begins at 128° C., and the anhydrate is stable between 600° and 950° C. The double uranyl acetate with magnesium, $[Mg\{UO_2(CH_3CO_2)_3\}_3]Na$, is of variable composition, with between 6 and 9 molecules of water (6·5 molecules at 20° C.), but is stable to 91° C. Losses of water and acetic acid occur simultaneously and become explosive above 275° C.; the residue is $0{\cdot}5\ U_2O_7Na_2.U_2O_7Mg$, is stable between 360° and 745° C., and is independent of the original precipitate. The analogous compound with zinc also contains between 6 and 9 molecules of water and is stable up to 75° C., the anhydrous range being only between 118° and 125° C. Loss of acetic acid then occurs up to 360° C. when the double pyrouranate, $0{\cdot}5\ U_2O_7Na.U_2O_7Zn$, which is stable below 674° C., is formed. The triple bismuth, cerium, and sodium nitrite, $6NaNO_2.9CsNO_2.5Bi(NO_2)_3$ is stable between 160° and 670° C.

Ammonium—The chloride is anhydrous between 40° and 126° C., but sublimation begins slowly at 110° C. Decomposition of the platinichloride, $(NH_4)_2PtCl_6$, begins at 181° C. and becomes explosive above 276° C. Between 407° and 538° C. a constant weight of platinum is obtained. The platinum is superficially oxidised to PtO at 607° C., but reverts quantitatively to platinum metal above 811° C.

Potassium—The chloride is anhydrous between 219° and 813° C. The anhydrous perchlorate is stable between 73° and 653° C., loss of oxygen occurring above that range. The pure sulphate is obtained between 408° and 880° C., no apparent sublimation occurring above 800° C. The cobaltinitrite is precipitated in a hydrated form, but there is no evidence of a stable monohydrated or anhydrous salt for use as a weighing form. Potassium per-rhenate, $KReO_4$, is stable between 54° and 220° C., but decrepitation begins at that temperature. The platinichloride can be weighed below 270° C.; decomposition occurs at higher temperatures, but is not complete even at 880° C. The chloro-6-nitro-5-toluene *m*-sulphonate, $CH_3.C_6H_2.(Cl)(NO_2).SO_3K$, is stable to 278° C.; explosion occurs at 345° C. The acid tartrate is stable and anhydrous to 200° C., but chars at higher temperatures. The picrate is stable to 217° C., but explosion does not occur until 310° C. The dipicrylaminate can be dried up to 220° C. without fear of explosion.

Rubidium—The anhydrous chloride is obtained between 88° and 605° C. The perchlorate is anhydrous between 95° and 343° C., decomposition beginning slowly at that temperature and becoming explosive at about 473° C. The sulphate, prepared from rubidium chloride and sulphuric acid, is dehydrated at 76° C. and is then stable to 877° C., with no apparent sublimation; when prepared from the acid carbonate no stable compound is formed even at 880° C.; this behaviour appears to be due to the formation of acid sulphates. The hydrated cobaltinitrite decomposes in complex fashion, constant weight being attained between 283° and 550° C., but the mixture of cobalt oxide, rubidium nitrate, and sodium nitrate formed is of variable composition. The stannichloride, Rb_2SnCl_6, is stable to 341° C. and loss of chlorine and stannic chloride occurs between 407° and 650° C., the residual mixture of rubidium chloride and stannic oxide of variable composition being stable between 650° and 812° C. The platinichloride is stable below 674° C., decomposing slowly up to 1000° C. into platinum and into rubidium chloride, which sublimes slowly. M. E. DALZIEL

Electro-Oxidation of Quadrivalent Uranium. R. H. Betts (*Canad. J. Res.*, 1948, **26**, **B**, 441–455)—The effect of various factors on the current efficiency of the electrolytic oxidation of quadrivalent uranium at a platinum anode has been studied.

The apparatus consists of two 100-ml. beakers serving as anode and cathode compartments, respectively, connected by a removable U-tube. A rectangular sheet of platinum, 3·0 sq. cm. in area, which can be rotated in one of the beakers at 300 to 500 r.p.m., serves as anode. A stationary platinum cathode is used and the anode potentials are measured with reference to a saturated calomel electrode connected to the anode compartment by means of a second U-tube filled with perchloric acid. Two 6-v. batteries connected to the electrodes through a rheostat provide the current, which is measured by a milliammeter in the circuit.

Procedure for measuring current efficiencies—Pipette suitable volumes of 1·0 N perchloric acid into the two beakers, and fill the U-tube with the same acid. Adjust the applied potential to give the required current density and then add a known volume of a uranous sulphate solution in 1·0 N perchloric acid. After the oxidation has proceeded for a measured time, determine the quadrivalent uranium remaining in the anode compartment by titration with potassium permanganate.

The current efficiency increases linearly with increasing uranium concentration. It decreases rapidly at first and then more slowly with increasing current density. J. G. WALLER

Microchemical

New Technique for the Micro-determination of Iron. L. Deibner (*Bull. Soc. Chim.*, 1948, 615–629M)—A detailed re-investigation of the iodimetric method has been made. In order to establish precisely the conditions found to be most satisfactory the iron is first precipitated as hydroxide and is redissolved in hydrochloric acid of definite concentration. The following factors influencing the accuracy of the method have been studied: type of filter, washing of the precipitate, concentration and volume of acid, use of carbon dioxide to eliminate dissolved oxygen, quantity of potassium iodide, and duration of the reaction. Starch mucilage (0·2 per cent.) is used as indicator and, with the very dilute solutions employed, the end-point is not a sharp change from blue to colourless, but is preceded by a succession of colours, *viz.*, blue, purple, red, violet, rose and yellow. This phenomenon is the more pronounced the greater is the concentration of potassium iodide and, even at the concentration of iodide employed, careful observation of the end-point is necessary.

Reagents—Strong emphasis is placed on the purity of all reagents, and the water used for most of the solutions is previously boiled. (1) Potassium iodide solution containing 1 g. in 1 ml. If this solution is yellow, decolorise it with $N/75$ or $N/150$ sodium thiosulphate; if it is colourless, add a slight excess of 0·01 N iodine and decolorise with thiosulphate. (2) Potassium iodide solution, 3 per cent., prepared in the same manner. (3) $N/75$ Sodium thiosulphate, prepared according to Hanes (*Biochem. J.*, 1929, **23**, 99) and kept in an amber glass bottle connected to the micro-burette through a syphon covered with black paper. Standardise this solution on a solution of 0·4 to 0·8 g. of potassium iodate per litre, using 3 ml. of the iodate solution, 3 ml. of 3 per cent. potassium iodide solution, and 3 ml. of 3 per cent. acetic acid. Allow this mixture to stand for 3 min., add 0·6 ml. of starch mucilage, and titrate with the thiosulphate solution.

Procedure—Use preferably a cylindrical 50-ml. vessel with a ground-glass stopper. The iron solution should be slightly acid and free from interfering elements and should contain 0·04 to 1 mg. of Fe as ferric chloride in 20 ml., the volume taken for analysis. Add 2 to 2·5 ml. of diluted hydrochloric acid (1 + 2), a few drops of hydrogen peroxide (110 volumes) and two glass balls, cover with a watch-glass, and boil for 1 min. Add about 0·5 g. of ammonium chloride or sulphate and a large excess of concentrated aqueous ammonia solution, keep at 50° to 60° C. for 30 to 45 min., then allow to stand for 2 to 3 hr. Separate the ferric hydroxide by means of a sintered-glass immersion filter, porosity 4, immersing the filter to the minimum extent. Connect the filter to the pump through a filter flask and fit a stopcock on a T-tube to allow regulation of the reduced pressure. Wash the vessel and filter with 150 to 200 ml. of 0·5 per cent. ammonium chloride or sulphate solution at 70° to 80° C. and then with about 75 ml. of hot water. Remove the water from inside the filter with a rubber-tipped capillary tube connected to the pump and dry the filter by aspiration without removing it from the precipitating vessel. Measure into the vessel 10 ml. of dilute hydrochloric acid (4 g. per litre) and warm it. Connect a tube containing a plug of cotton wool to the end of the filter and suck the warm acid through the filter-plate several times. Then connect the tube to a small pump and eject the liquid into the vessel. Use 5 ml. more of the dilute acid to wash the outside of the filter and to wash the plate twice from the inside, introducing it through the tube and ejecting it through the plate. From this point conduct a parallel blank experiment starting with 15 ml. of dilute hydrochloric acid (4 g. per litre). Pass through the liquid for 5 min. a current of pure carbon dioxide. Raise the gas delivery tube above the liquid surface without interrupting the gas current, add 1 ml. of potassium iodide solution (1 g. per ml.), mix, stopper, and allow to stand for exactly 5 min. Titrate with $N/75$ or $N/150$ sodium thiosulphate from a 2-ml. micro-burette adding 0·8 ml. of starch mucilage when the colour becomes pale yellow. Stir the solution after each addition, and towards the end of the titration add the thiosulphate in quantities of 0·005 ml. at intervals of several seconds. The end-point (*vide supra*) is best observed against a piece of opal glass and it should be checked after a final interval of 30 to 40 sec.

Results of a large number of determinations show errors varying between −5·6 and +7·8 per cent.

W. C. Johnson

Physical Methods, Apparatus, etc.

Crystallographic Data, Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 491–492)—The crystallography of ethylenediamine D-tartrate and hemihydrated dipotassium hydrogen tartrate is set out under the headings listed in the first publication of the series (*Ibid.*, 1948, **20**, 275; Abstr., *Analyst*, 1948, **73**, 579).

M. E. Dalziel

Crystallographic Data. Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 593–595)—Data are given for *p*-methyl aminophenol sulphate (Metol, Elon) and *p*-aminoazobenzene (Aniline Yellow S) under the headings listed in the first paper of the series (*cf.* preceding abstract).

M. E. Dalziel

Accelerated Method for Measuring the Penetration of Water in Insulation Board. W. A. Wink and J. A. Van den Akker (*Paper Trade J.*, 1948, **127**, Sept., *T.A.P.P.I. Sect.*, 371–376)—Grant's method (*Analyst*, 1935, **60**, 60) for rendering water penetration through paper visible by use of a fluorescent indicator, is extended to boards.

Procedure—Cut a disc of sample (minimum diameter 6 in.), seal the edges with wax, and place it horizontally on water at 130 ± 0·5° F., so that the lower surface is immersed to a depth of ⅛ in. After a period appropriate to the nature of the sample (1 to 2 hr.), saw the specimen into four quarters, at once sprinkle the cut edges with powdered Uranine-B (using a pepper-box, covered with an 80- to 100-mesh wire), and examine them in filtered ultra-violet light. The light yellow fluorescence of the damp dye shows the depth to which penetration has occurred, and enables it to be measured with an accuracy to 1/16 in. Experimental data are tabulated, and are in agreement with values obtained from direct measurements of the distribution of the water in the board.

J. Grant

Spectrographic Determination of Sodium in a Silica-Alumina Catalyst. J. B. Marling (*Anal. Chem.*, 1948, **20**, 299–300)—Sodium is determined in a silica - alumina catalyst by a simple and rapid spectrographic method. The sample is placed in the crater of a graphite electrode and is excited by a 2300-volt, 2·5-amp., A.C. arc. The counter electrode is of high purity copper, and copper lines are employed as internal standards. Each sample is analysed three or four times and the results are averaged, giving a final result sufficiently accurate for industrial control and more accurate than the average chemical method for low percentage ranges. The time taken for an analysis is 30 to 50 min. The method has been tested by comparison with chemical analyses and the average deviation in the specification range (0·02 per cent. of Na_2O or less) is 0·002 per cent. of sodium oxide.

Modifications to the technique employed are suggested as a means of improving its accuracy, but have not been checked experimentally.

H. R. Clayton

Emission Spectroscopy in an Oil Laboratory. R. G. Russell (*Anal. Chem.*, 1948, **20**, 296–298)—The applications of emission spectrographic analysis in an oil laboratory are described. For general qualitative work the D.C. arc is used and the samples, in the form of oils, liquids or organic residues, are either evaporated to dryness or ignited and burned. The resulting material is mixed with graphite powder and loaded into a cavity in the end of a graphite or copper rod that is then arced. For qualitative analysis of surface films a series of low-powered, short-duration, alternating-current spark discharges is employed with a graphite rod as counter electrode. The limit of detection by this method is 0·000001 g. of material. As an example of quantitative methods the analysis of silica - alumina catalysts is described (*cf.* preceding abstract). Standard samples are prepared by addition of known amounts of sodium chloride to samples of a catalyst that is substantially free from sodium and these standard samples are included on each analytical plate. The standards and samples are prepared for analysis by mixing 1 g. of the material with 2·2 g. of pure graphite powder and 50 mg. of lithium carbonate, which is used as an internal standard. The resultant powder is briquetted and used as the lower electrode of an alternating-current spark discharge. The upper electrode is a graphite rod of ¼-in. diameter, with a hemispherical conical tip. Four analyses are made of each sample and the average of these results is more accurate than the routine chemical determinations. The analysis of brines, a method of analysis of oil additives, and a technique for dealing with small quantities of solutions, are briefly mentioned.

H. R. Clayton

Study of Reversibility of Processes at the Dropping Mercury Electrode by Changing Discontinuously the Polarising Voltage. M. Kalousek (*Coll. Czech. Chem. Comm.*, 1948, **13**, 105–115)—The reversibility of a polarographic reaction can be investigated by continually altering the potential applied to the cell. A simple electric circuit is described by which the potential of the dropping mercury electrode can be alternated five times per sec. between two potentials that are from 60 to 400 mv. apart. At the same time, the potential of the electrode is gradually increased as in normal polarographic procedure. If the electrode reaction is a reversible reduction, an anodic current is obtained when reduction occurs at the more negative potential and not at the less negative. If the reduction is irreversible, only the cathodic current is obtained. Analogous phenomena are exhibited by oxidation reactions.

J. G. Waller

Reviews

British Chemical Nomenclature. By A. D. Mitchell, D.Sc., F.R.I.C. Pp. viii + 156. London: Edward Arnold & Co. 1948. Price 21s.

Chemical nomenclature is not one of the most popular of pastimes, at all events in this country, but there are signs that the general apathy of chemists to the subject is disappearing; witness the fact that one of the largest lecture theatres in London could barely accommodate the audience that assembled to hear Dr. G. M. Dyson lecture on his new system of notation. It is to be hoped that Dr. Mitchell's book will meet with the same degree of interest; for the conventional method of describing chemical substances, which forms its subject, will be in use for a long time to come, even though some system of notation is used for indexing, and the official adoption of any system will be useless unless individual chemists take the trouble to learn the rules and then apply them intelligently. With the publication of "*British Chemical Nomenclature*," there is no longer the excuse that it is impossible to find out what the rules are.

Even the most inexperienced of chemists, when looking up references to a particular compound that can be described quite correctly in several different ways, must appreciate how much time can be saved if he knows, for example, the order in which the substituents should be listed or the recognised name of the fundamental ring system from which the substance is derived. It is perhaps not so well known, that in spite of the efforts of the International Union of Chemistry, considerable discrepancies still exist in the methods adopted in different countries in the naming and numbering of ring systems, for instance. Dr. Mitchell deals with these and many other difficulties, some of which are too often dismissed as worthless trifles. Do we, for example, use Greek and Latin numerical prefixes correctly? Do we know when to use "primed" numbers? How many ways are there of misusing that over-worked prefix "bi"? When is "naphtho-" correct and "naphtha-" incorrect? Should the well-known plant hormone be called indoleacetic acid or indolylacetic acid? Here, indeed, is a wealth of material for a chemical "quiz"!

Not that Dr. Mitchell solves all these problems for us, for as he says in the preface: "It is impossible to lay down rules to cover even all the simpler compounds, and it is necessary to allow considerable latitude in the use of trivial names, but this volume aims at a statement of conventions adopted in the *Journal of the Chemical Society*. The view taken by successive editors of that journal has usually been that, nomenclature being such a complex subject, a name that is free from any possibility of ambiguous interpretation is the primary requisite, and if two or more names fulfil this condition they may be equally acceptable provided they do not infringe any usages." He does, in fact, achieve a very nice balance between the rigidity necessary to prevent chaos and the flexibility that is a pre-requisite to international (and sometimes intranational) agreement.

Part I of "*British Chemical Nomenclature*" is an account of the general principles of nomenclature, and Part II describes their application to inorganic chemistry. This second part is largely an exposition of the rules adopted by the International Union of Chemistry, and some of the conclusions reached are revolutionary. Henceforth, for example, sodium hydrosulphite ($Na_2S_2O_4$) must be called sodium dithionite, and potassium metabisulphite ($K_2S_2O_5$) potassium pyrosulphite. But the biggest shock of all is provided by the statement that the name "sodium bicarbonate" is no longer permissible and should be replaced by "sodium hydrogen carbonate." The sections on co-ordination compounds and complex salts (borates, silicates, tungstates, etc.) make less startling innovations and the systems proposed are both simple and logical—qualities that do not always go together.

Part III, forming three-quarters of the book, deals with organic chemical nomenclature, and recounts the difficulties introduced by the adoption of different systems or by the absence of any system, and the attempts made to resolve these difficulties by Richter, Stelzner, the Ring Index, *Chemical Abstracts* and the International Union of Chemistry. The author shows great skill in piloting his readers through these very troubled waters, and all chemists engaged in the preparation of new substances should make themselves thoroughly familiar with the relevant sections of this book before presuming to name any new compound. They will thereby avoid the grievous sin of burdening the literature with incorrect names, which are almost impossible to eradicate once they have come into general use. It is valuable to have the various conventions available in such a convenient form with the benefit of Dr. Mitchell's expert comments. The book is up-to-date, and includes the rules recently proposed for indicating the orientation of sugars and amino acids and a description of that most useful device, the "oxa-aza convention."

Apart from its value to chemists actively engaged in the synthesis of new compounds, and to those responsible for editing scientific papers and reports, "*British Chemical Nomenclature*" will be found useful by the general chemist for purposes of reference. It has obviously been most carefully edited, no easy matter in view of the number of graphic formulae that it contains, and is well indexed. The excellent binding also deserves a word of praise.

F. A. Robinson

SPECTROSCOPY AND COMBUSTION THEORY. By A. G. Gaydon, D.Sc. Second Edition. Pp. xii + 242. London: Chapman & Hall, Ltd. 1948. Price 25s.

The mechanisms of the various combustion phenomena are still imperfectly understood both from the physical and from the chemical standpoint. The theories advanced in this field are necessarily based on evidence that is not always direct and the interpretation of which is therefore often largely conjectural. Progress is to a certain extent restricted by the lack of observations, a fact that is not surprising in view of the wide range of the subject, and its comparative infancy. Information is now, however, being rapidly gathered, and a growing number of communications are appearing in the literature on various aspects of combustion. This second edition of Dr. Gaydon's book, published six years after the first, is therefore appropriate and welcome.

The opening chapter, which introduces the reader to an outline of molecular spectra, remains unchanged. This is to be regretted, as the treatment is of necessity so brief that it seems insufficient to enable the non-specialist to appreciate much of the theoretical development which follows. After a further general consideration of the appearance and the interpretation of various spectra, and an expanded survey of the experimental problems, there follow chapters devoted to various flames. The flames considered are those of hydrogen, the hydrocarbons, carbon monoxide, inorganic elements and compounds, cool and atomic flames and explosions. For each of these, details are given of the appearance of the spectra and an assessment is made of the significance of the various systems observed. The four plates which illustrate the features discussed in these and later chapters reproduce nineteen spectra, in place of the original ten, and are better laid out, with the accompanying descriptions on the opposite pages.

The ninth chapter, a new one, is concerned with continuous spectra and the rôle of atomic oxygen in flames, the presence of which can be demonstrated by the appearance of a yellow-green continuum on addition of a small amount of nitric oxide to the flame gases. Dr. Gaydon has applied this test to several flames and discusses the bearing of the results on proposed combustion mechanisms.

The subsequent chapters make several approaches to the general subject: absorption spectra with special reference to slow burning and the internal combustion engine, the infra-red region with its practical difficulties, life-times of activated molecules and the author's theory of after-burning, the measurement and calculation of flame temperatures and dissociation energies. Many of these ideas are interwoven and demonstrate well the many ways in which reactions in flames are being investigated. Finally, under the heading "Kinetic Spectroscopy," Dr. Gaydon indicates lines of study which he believes will prove most fruitful in analysing flame reactions by the spectroscope. As with general chemistry, the original observations were largely qualitative, but now more quantitative data are required in all regions of the spectrum.

There are six appendixes containing much useful information on absorption and emission spectra and atomic and molecular energy levels and also a bibliography comprising 301 references. The book is printed on better paper than the original war-time edition, and revision and expansion of the text have enlarged it by about fifty pages. In all, it is a readable book which fills a gap, and fills it well.

ROLAND A. FINCH

APPLIED CHEMISTRY: A PRACTICAL HANDBOOK FOR STUDENTS OF HOUSEHOLD SCIENCE AND PUBLIC HEALTH. By C. KENNETH TINKLER and HELEN MASTERS. Second Edition, revised and reprinted. Vol. II: FOODS. Pp. x + 284. London: The Technical Press Ltd. 1948. Price 16s. net.

This book, with its companion first volume, is intended primarily for students taking the course in Applied Chemistry for the degree of B.Sc. (Household and Social Science) of the University of London. Its scope is indicated by its chapter division—Milk; Edible Oils and Fats; Carbohydrate Foods; Raising Agents; Meat and Meat Extracts; Vinegar, Fruit Juices and Vegetable Acids; Beverages; Preservation of Food; Poisonous Metals in Foods; Cooking of Food; Condiments; Calorific Value of Foods.

The text is largely concerned with the examination and analysis of foods, and the methods described are mainly those in routine use in a public analyst's or food manufacturer's laboratory. Objection must be raised to the inadequate treatment accorded to the determination of the freezing-point of milk by means of the Hortvet cryoscope. Although references to reports of the work of Elsdon and Stubbs in *The Analyst* are given, a method so important as this is worthy of fuller description, even in an elementary textbook. The experimental details are omitted, there is no indication of the range of the freezing-point of normal milk, there is a suggestion, perhaps unintentional, that the Hortvet cryoscope and the Beckmann cryoscope are interchangeable, and the statement that supercooling must be avoided is surprising when the principle of the Hortvet apparatus is the attaining of a definite and reproducible degree of supercooling before freezing.

Where necessary the theoretical bases of the methods are lucidly explained and the student will acquire a good knowledge of routine food analysis, but he is not always provided with the correct means of interpreting his results, especially if he is interested in the legal aspect of food examination. He is not informed that the legal standard for milk is a presumptive one, and there is a suggestion on page 168 that the inclusion of cereal filler in sausages is adulteration. The statement that preservatives are not allowed in butter and margarine requires qualification, since margarine manufactured under Government licence now contains boron preservative. Again, starch added to mustard is not necessarily an adulterant.

Admittedly it is impossible in a book of this kind to guide the student through the mass of variable and transient war-time and post-war-time legislation affecting food composition, but mention might have been made of the standards established by the Ministry of Food for such materials as Baking Powder (otherwise afforded very full treatment in the book), Mustard, Self-Raising Flour and Preserves, especially since some of the Statutory Rules and Orders establishing these standards describe the method by which the food is to be examined.

In the section on pasteurised milk the test with *p*-phenylenediamine for the destruction of peroxidases is described, but there is no mention of the more recent and more important test for adequate pasteurisation depending upon destruction of the phosphatase by heat.

In spite of these deficiencies the book can be recommended as a comprehensive practical introductory course for the student of the application of chemistry to the examination of food, especially if he consults the original papers cited in the text, and supplements his reading with a leisurely browsing through Bell's "*Sale of Food and Drugs*," a book included in the bibliography at the end of the volume.

Type and paper are good and the binding is firm and attractive. Of the few typographical errors the only one needing mention is the appearance of 10° (degrees) for 10^0 (10 to the power of nought) on page 185.

A. O. JONES

ORGANIC ANALYTICAL REAGENTS. By F. J. WELCHER, Ph.D. Vol. III, pp. xi + 593. Vol. IV, pp. xiii + 624. New York: D. Van Nostrand Company Inc. London: Macmillan & Co. Ltd. 1947 (Vol. III), 1948 (Vol. IV). Price, each volume, 44s. net.

The plan of this work has already been described in the detailed review of the first two volumes (*Analyst*, 1948, **73**, 420). The third volume, unlike the others, is divided into three parts, namely, Heterocyclic Nitrogen Compounds, the Oximes and Acidic Imino Compounds. The book begins with a chapter on pyridine and its derivatives, and the analytical utility of the parent substance, as well as the thoroughness of the presentation, is strikingly illustrated by the 43 pages of subject-matter and the list of 170 references. Also included in this first part is an important chapter on dipyridyl and related compounds. The second part begins with a discourse on the analytical applications of the dioximes, and herein one finds a monograph concerning the use of dimethylglyoxime in which 303 references are cited. This section of the book also includes, among others, chapters on *iso*nitroso compounds, nitroso phenols, miscellaneous oximes and cupferron. The high light of the third part is the exhaustive study, both theoretical and practical, of diphenylthiocarbazone, in which 212 references are recorded. Many data have been published about the conditions under which metallic complexes of this reagent are formed and many procedures for attaining various degrees of specificity have been proposed. The mass of original literature devoted to this theme is indeed a tortuous maze, and the well-arranged account presented will certainly prove to be of great value.

Perhaps the most important substance discussed in Vol. IV is sodium diethyldithiocarbamate, which is one of six reagents assembled in a chapter devoted to the dithiocarbamates. While all the essentials of its use are given, together with 69 references, the transcription of the procedure due to L. A. Haddock and N. Evers (*Analyst*, 1932, **57**, 495) might advantageously have included the useful table of colour values in terms of Lovibond units, given in the original paper. As with the previous volumes, some of the monographs hardly seem to warrant their allotted space; for example, the one on sodium tauroglycocholate for which the only recommended use is its addition to reaction mixtures of 8-hydroxyquinoline and magnesium salts to prevent the precipitate from adhering to the sides of the beaker. However, it is more important to mention that the text concludes with 267 pages of invaluable information concerning the application of dyestuffs to analytical chemistry.

Summarising, it is the considered judgment of the reviewer that these two volumes, like their predecessors, ought to be added to every chemical library throughout the world.

N. L. ALLPORT

Notice

MICROCHEMISTRY GROUP

Meeting in Belfast, May 10th–11th, 1949

A MEETING of the Microchemistry Group, jointly with the Belfast and District Sections of the Royal Institute of Chemistry, the Chemical Society and the Society of Chemical Industry will be held on the above dates.

On the afternoon of Tuesday, May 10th, there will be a tour of the Biochemistry, Chemistry and Agricultural Chemistry Departments of the Queen's University, Belfast. This will be followed by a meeting in the evening at the University, at which the following papers will be read.

"Some Microchemical Problems Associated with Plant and Animal Nutrition," by Stewart McConaghy, B.Sc., M.Agr., A.R.I.C., Dept. of Agricultural Chemistry, Queen's University of Belfast.

"The Micro-Determination of Oxygen in Organic Compounds by Reduction with Carbon," by W. T. Chambers, B.Sc., Ph.D., A.R.I.C., of The British Rubber Producers' Research Association, Welwyn Garden City, Herts.

On Wednesday, May 11th, there will be a coach tour of the famous Antrim Coast Road.

Address of the Honorary Secretary of the Group

From now on, the address of the Hon. Secretary of the Microchemistry Group, Mr. Donald F. Phillips, A.R.I.C., is 10, Richmond Road, Blackpool, N.S., Lancs.

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, February 2nd, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following papers were presented and discussed:—"A Rapid and Accurate Volumetric Method for the Determination of Silica," by H. N. Wilson, F.R.I.C.; "The Analysis of Nylon and Related Polymers," by M. Clasper, A.R.I.C., and J. Haslam, M.Sc., F.R.I.C.; "The Quantitative Separation of Beryllium and Aluminium," by W. C. Coppins, M.Sc., A.R.I.C.

NEW MEMBERS, ELECTED FEBRUARY 2nd, 1949

Alma Aranshew Christie, A.R.I.C.; Sam Collett, Ph.C., M.P.S.; Nathan Goldenberg, M.Sc. (Lond.), F.R.I.C.; Miss Audrey Mabel Jones; Oswald Hilton Keys, M.Sc. (N.Z.), A.R.I.C.; Norman Kirby, B.Sc. (Lond.), A.R.I.C.; Roy Albert Knight, B.Sc. (Lond.); James Ravantós, M.D.; Edward John Rolfe, B.Sc. (Lond.), A.R.I.C.; Edwin Weedon Smith, B.Sc. (Lond.), A.R.I.C.; George Alexander Stewart, B.Sc. (Lond.); Ronald Wellwood, B.Sc. (Q.U.B.).

ANNUAL GENERAL MEETING

The Annual General Meeting of the Society was held at 3.30 p.m. on Wednesday, March 9th, 1949, in the meeting room of the Royal Society, Burlington House, London, W.1. The chair was taken by the President, Mr. Lewis Eynon. The Financial Statement for 1948 was presented by the Hon. Treasurer and approved, and the Auditors for 1949 were appointed. The Report of the Council for the year ending March, 1949 (see pp. 157–162) was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year.

President—George Taylor, O.B.E., F.R.I.C.

Past Presidents serving on the Council—F. W. F. Arnaud, Lewis Eynon, E. B. Hughes, G. Roche Lynch, S. E. Melling, and G. W. Monier-Williams.

Vice-Presidents—C. A. Adams, H. E. Cox, J. R. Nicholls and, *ex officio*, J. G. Sherratt (Chairman, North of England Section), and J. Sword (Chairman, Scottish Section).

Hon. Treasurer—J. H. Hamence.

Hon. Secretary—K. A. Williams.

Other Members of Council—N. L. Allport, R. C. Chirnside, J. F. Clark, D. C. Garratt, J. G. A. Griffiths, E. T. Illing, J. King, J. E. Page, C. J. Regan, F. A. Robinson, N. Strafford, A. M. Ward and, *ex officio*, Arnold Lees (Hon. Secretary, North of England Section), and R. S. Watson (Hon. Secretary, Scottish Section).

After the business outlined above had been completed the meeting was opened to visitors, and the retiring President, Mr. Lewis Eynon, B.Sc., F.R.I.C., delivered his Presidential Address (see pp. 163–167).

NEW MEMBERS, ELECTED MARCH 9th, 1949

David George Allen, B.Sc. (Lond.), A.R.I.C.; Charles Gerald Barlow, M.A. (Cantab.), A.R.I.C.; John Brinley Bowen, B.Sc. (Wales), A.R.I.C., Dip. Ed. (Wales); Eugene Henry William James Burden, B.Sc. (Lond.), A.R.I.C.; Brian Crossley Christian, B.Sc., Ph.D. (Liv.), F.R.I.C.; Walter Charles Coppins, M.Sc. (Lond.), A.R.I.C.; Ernest Walter Courts, B.Sc. (Birm.), A.R.I.C.; Stuart Herbert Henry Davison, B.Sc. (Lond.), A.R.I.C.; George Drewery, B.Sc. (Lond.); Edward Alfred Elsbury, F.R.I.C.; Robert Alexander Fraser, B.Sc. (Lond.), A.R.I.C.; Jack Arthur Gilby, B.Sc. (Lond.), A.R.I.C.; Maurice Speakman [illegible] Green, B.Sc. (Lond.), A.R.I.C.; Donald Hanson, B.Sc. (Lond.), F.R.I.C.; Laurence John Arth [illegible] ur Haywood; Leslie Frank Hewitt, B.Sc., Ph.D. (Lond.), F.R.I.C.; Leslie Charles Horace Halye [illegible] day, B.Sc. (Lond.), A.R.I.C.; Robert Knox, A.R.I.C.; Charles Michael Lavender, B.A. (Oxon.); David Evan Lightfoot, B.Sc. (Birm.), A.R.I.C.; James Kidd McLellan, M.A., B.Sc. (Glas.), A.R.I.C.; Maurice Pereira Mendoza, B.Sc. (Lond.), A.R.C.S.; Francis Albert Oliver, B.Sc. (Lond.), A.R.I.C., A.C.T.C. (Birm.); Victor Harold Parks, F.R.I.C.; Ernest Pedley, M.Sc. (Manc.), Ph.C., A.R.I.C.; William Patrick Pepper, M.Sc. (Liv.), F.R.I.C.; Giulio Richard Primavesi, B.A. (Cantab.); Norman Frank Rapps, B.Sc. (Lond.), A.R.I.C.; Edward Rogers, A.R.I.C.; Jack Palmer Savage, B.Sc. (Liv.), A.R.I.C.; Edward John Skerrett, A.R.I.C.; William Cuthbert John Smith, A.R.I.C.; William Arthur Stephens, A.R.I.C.; Alfred McMullon Taylor, B.Sc., Ph.D. (Lond.), F.R.I.C.; Edmund Alan Taylor, B.Sc. (Lond.); Ivan Waide, A.R.I.C.; William Ward; Richard John Whiffin, Ph.C.; Arthur William Williams.

DEATHS

We regret to record the deaths of

Charles Thomas Bennett.
John Henry Coste.
William Rhys Davies.
Thomas Wentworth Glass.
James Hendrick.
Rowland Williams.

Anniversary Dinner

In the evening of the day of the Annual General Meeting the Society held a Dinner at the Trocadero Restaurant, Piccadilly, London, W.1, to celebrate its seventy-fifth anniversary.

The members and guests, numbering 140, were received by the President, Mr. Lewis Eynon, and Mrs. Eynon. The President afterwards took the chair at the Dinner.

The guests of the Society and of the President included: Sir Robert Robinson, M.A., Hon.D.Sc., Hon.LL.D., Hon.D.Pharm., F.R.I.C., President of the Royal Society, and Lady Robinson; Professor E. K. Rideal, M.B.E., M.A., Ph.D., D.Sc., F.R.S., Chairman of the Chemical Council; Dr. G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.R.I.C., President of the Royal Institute of Chemistry, and Miss Roche Lynch; Dr. G. M. Bennett, C.B., B.A., Ph.D., M.A., Sc.D., F.R.I.C., Government Chemist; H. W. Cremer, Esq., C.B.E., M.Sc., M.I.Chem.E., M.Inst.F., F.R.I.C., President of the Institution of Chemical Engineers; Dr. R. P. Linstead, C.B.E., M.A., Ph.D., D.Sc., F.R.I.C., Vice-President of the Chemical Society; H. N. Linstead, Esq., M.P., O.B.E., Ph.C., Joint Secretary of the Pharmaceutical Society.

After the loyal toasts had been honoured, Professor E. K. Rideal proposed the toast of the Society. He said he was not sure why he had been chosen to do so when others more worthy were present, but perhaps it was because his father had at one time been president of the Society. He congratulated the Society on its age and growth. As Chairman of the Chemical Council he was particularly interested in this Society and the Faraday Society—the two most recent member bodies of the Chemical Council—and wondered which of them would reach the 2000 membership mark first.

It was not only the most important Society dealing with analytical chemistry, but was also a publishing body, whose journal, *The Analyst*, was unique in the chemical world. The activities of the Society covered and recorded a very wide range of specialist subjects, and for that reason, in days in which the specialist was becoming more than ever important, it was doing work of real value.

He had been astonished by the relatively small place that instruction in analysis occupied in the curricula of our leading chemical teaching institutions, and hoped that this condition of affairs would be speedily remedied.

The President, replying to the toast, thanked Professor Rideal—a distinguished son of a distinguished Past President of the Society—for his kind remarks on the work of the Society. The work was of an unobtrusive character, but none the less very important. Its complexity and many-sidedness came as a surprise to many people. He recalled a conversation he had had with a friend to whom he mentioned that he had to attend a meeting of the Society. The friend remarked: "But I didn't know you were a Public Analyst." He replied that he was not; he was one of the Other Analytical Chemists. His friend asked: "But is analytical chemistry the only thing you have to talk about?" to which he replied that "the more we talk about analytical chemistry, the more there is to say." It was indeed true, for new problems were always arising, new methods were always being discovered and known methods improved. The progress and development of analytical chemistry had kept pace with the demands made upon it. Thanks to Public Analysts, gross adulteration of food had almost disappeared. But Public Analysts were now presented with much more difficult and subtle problems than formerly; for example, the determination of certain substances present only in traces had become of great importance. Here the work of the Other Analytical Chemists had helped very much. It was the business of many of the Other Analytical Chemists to see that accidental contamination of food did not occur. There was now hardly any industry that did not rely, for its proper control, directly or indirectly, on analytical chemists.

For many years past the Society had had Local Sections in the North of England and in Scotland. These had been a valuable means of fostering mutual help amongst members in those areas. Within the past few years, Subject Groups had also been formed, to promote the development of microchemical, physical and biological methods of analysis, respectively. Those Groups had more than justified their formation, familiarising the Society with new and sometimes rather astonishing techniques, and incidentally with some strange terminology. It might almost be said that one of the differences between the Sections and the Groups was that the Sections spoke the same language as the parent Society, but the Groups seemed to be developing their own languages. The Society was being faced with the necessity of developing a language that would be intelligible to all.

Sir Harry Jephcott, who proposed the toast of the Guests, coupled with the names of Mr. H. W. Cremer, C.B.E., President of the Institution of Chemical Engineers, and Mr. H. N. Linstead, M.P., Joint Secretary of the Pharmaceutical Society, said that the guests included many eminent persons, and the Society did most heartily welcome them. There were the President of the Royal Society, the President of the Royal Institute of Chemistry, the Chairman of the Chemical Council, and a Vice-President of the Chemical Society. It might seem presumptuous to comment on them, but he would like to mention one observation made to him when on a recent visit to the United States. He had remarked, on the subject of research, that this country had not at its disposal the large number of chemists available in the States, to which the reply was made: "No, but you have some very distinguished ones, in fact, the most distinguished organic chemist in the world, Sir Robert Robinson." As an industrial chemist himself, he was particularly pleased to welcome the President of the Institution of Chemical Engineers. He could not too highly praise the contribution that had been made by that body and its President in helping to raise the productive output of this country. In welcoming Mr. Hugh Linstead, Secretary of the Pharmaceutical Society, he experienced a personal gratification, not unmixed with hopes for the future, at the recollection of most excellent lunches that for several years past he had been able to attend as an Honorary Auditor of that Society. He greatly admired the skill, tact, and diplomacy with which Mr. Linstead was able to conduct his Council into what he considered the right path of life. On a recent occasion the same tact appeared to have induced a very large number of people to enter the polling booth and put a cross in the right place. He would like to refer also to the Government Chemist, who represented the scientific officers of his own and several other Departments. His position was unique and represented a magnificent example of co-operation between Government Departments, industry and other organisations. It was significant that many of his scientific officers were well-known and very highly respected members of the Society. Sir Harry ended by extending a particular welcome to the ladies, who had added so greatly to the pleasure of the evening.

Mr. H. W. Cremer, President of the Institution of Civil Engineers, said he greatly appreciated the honour done to his Institution and to himself, in being selected, with Mr. Hugh Linstead, to reply on behalf of the guests. He did so with some embarrassment in the presence of some much more distinguished chemists, but he was relieved to feel that he was not regarded as a complete outcast from the chemical profession. He was, in fact, brought up in the orthodox chemical faith and passed the examinations of the Institute of Chemistry, which had not then its Royal status. He hoped he could say that since then his stay in a far country had not been spent in riotous living—war time abroad could hardly be so described. All the guests had greatly enjoyed the feast that had been prepared for them and the charming company, and they drank to the prosperity of the Society and its influence in the time to come.

Mr. H. N. Linstead, M.P., Joint Secretary of the Pharmaceutical Society, said he was pleased to have the honour of replying on behalf of the guests and the opportunity of thanking Sir Harry Jephcott and the President. He understood that the membership of the Society had now reached 1500, and wondered if as the cunning of mankind increased there became a greater need of Public Analysts. Many of the members of the Society were Public Analysts and many others were more or less associated with Government Departments, but he hoped there would always be independent analysts to whom the ordinary citizen could feel that he could turn for independent advice. He would like to congratulate Dr. Nicholls, the Chairman of the Publication Committee, and the Editors on the high standard maintained by *The Analyst.* It was one of the journals that came to his desk every month; he could not say that he read it, but he took his hat off to it. He would like also to pay a tribute to the late Dr. C. A. Mitchell, who made *The Analyst* part of his life's work. On the subject of published papers he wondered if it was possible to change the very common practice in which the authors at the end of a paper thanked their employers A and B for permission to publish. It seemed to him that the direction of the thanks would more appropriately be reversed. He thought that in one respect analysts were in a much more enviable position than many other practitioners; they had definite problems to solve and had the satisfaction of completing them and knowing they were finished. How different from the work of those in Parliament who, however carefully they analysed their problems, never seemed to get a final solution!

The President then proposed the toast of the President Elect, Mr. George Taylor. He had known him many years, having been a fellow student with him at college. It certainly did not then occur to either that they would be successive Presidents of the same Society. Mr. Taylor had the advantage of working for very many years with the late Dr. Bernard Dyer, and it would hardly be possible to imagine a better school for the training of a President. With that recommendation and the knowledge of Mr. Taylor's personal qualities, they could look forward with confidence to the maintenance of the prestige and welfare of the Society.

Mr. Taylor expressed his thanks to the President for his kind remarks and to the Society for the cordial way in which the toast had been received.

At the request of the President, Sir Robert Robinson made some concluding remarks. He expressed his pleasure at being made an Honorary Member of the Society. Analytical chemistry was a subject near to his heart. Referring to the projected International Conference on Analytical Chemistry, which it was proposed to hold in London in 1952, he sincerely hoped that it would be arranged and receive the necessary support from the powers that be. He was very interested in the subject of tuition in analytical chemistry at our universities, and was glad to know that it was one of the main topics dealt with in the President's address that afternoon. From the educative point of view he thought the fundamental principles of analysis—the use of the balance and the ordinary processes of analytical chemistry—were essential. The small amount of analytical work usually done by the student was to be deplored, and more analysis should be insisted on in the first years of a student's course. But he did not attach much educative value to the more specialised and complex apparatus, *e.g.*, apparatus that would do almost everything except write a paper.

Annual Report of Council: March, 1949

The roll of the Society numbers 1496, an increase over the membership of a year ago of 107.

Honours—The Council is glad to record that during the year the C.B. has been awarded to Dr. G. M. Bennett, the C.B.E. to Professor Alexander Findlay, Mr. B. C. Aston and Mr. C. A. Adams, the O.B.E. to Mr. George Taylor and the M.B.E. to Mr. G. W. Baker, Mr. R. C. Frederick, Mr. W. A. Godby and Dr. G. V. James. Mr. S. E. Melling has been awarded the honorary degree of M.Sc. by the University of Manchester. The Council offers its congratulations to the recipients of these honours.

Deaths—The Council regrets to have to record the death of the following members—

J. E. Byles
U. A. Coates
J. H. Coste
J. Davies
T. W. Glass
E. Halliwell
C. A. Hill
W. G. Leach
A. H. Mitchell
G. Rudd Thompson
S. R. Trotman
Rowland Williams

Byles, who died in his 59th year, was educated at Banham Grammar School and the University of Manchester. He entered the Government Laboratory, working from 1911 to 1926 mainly on the analysis of foods, fertilisers and feeding stuffs. For the next six years he was stationed at the Custom House, Liverpool, and for a further six years at the Custom House, London. He then came back to the Government Laboratory, where he was a Senior Principal Scientific Officer at the time of his death.

Coates joined the Society in 1920 and was for many years Joint Honorary Auditor of the North of England Section.

Coste was trained at Finsbury Technical College in 1888–91 and was a member of the staff of Augustus Voelcker from 1891 to 1894. He then joined the Chemical and Gas Testing Department of the London County Council, and when the main part of the work was transferred in 1912 to the Public Health Department, he became what is now described as the Chemist-in-Chief of the Chemical Branch. He retired in 1936. He became a Fellow of the Institute of Chemistry in 1896 and was a founder Fellow of the Institute of Physics. He served on the Councils of the Institute of Chemistry and the Society, and was for some years Honorary Secretary of the London Section of the Society of Chemical Industry. He was one of the first members of the Atmospheric Pollution Research Committee, now a constituent Committee of the Fuel Research Board, D.S.I.R. He joined the Society in 1907.

Davies was the last of the original members associated with A. Norman Tate who conducted a large analytical practice and a School of Chemistry in Liverpool. On the death of his colleagues he took over the firm of A. Norman Tate & Co. Unassuming and simple in his tastes, he had a profound knowledge of the chemistry and technology of oils and fats. In his youth he was an ardent cricketer, and all his life he was devoted to photography. He joined the Society in 1934.

Glass joined the Society in 1887 and served on the Council in 1917–18. He was for many years associated in practice with Mr. Edward Hinks.

Halliwell died in his 75th year. He was trained at the Yorkshire College, Leeds, becoming an Associate of the Royal Institute of Chemistry in 1894. He was then appointed an assistant to Thomas Fairley, Public Analyst for Leeds and the North Riding of Yorkshire. In 1897 he took charge of the laboratory of the West Riding Rivers Board. He became Chief Inspector and Chemical Adviser to the Ribble Joint Committee in 1902, retiring from that position in 1938. He joined the Society in 1897.

Hill joined the Society in 1906 and died in his 75th year. He was educated at Winchester, at the Pharmaceutical Society, at St. Thomas's Hospital and at King's College, London. From 1895 to 1896 he was analyst to A. S. Hill & Son and then directed the laboratories of Davy Hill & Son, Yate & Hicks, in Southwark. He was the prime mover in forming, in 1909, the British Drug Houses Ltd., becoming managing director and later chairman. He was Master of the Salters' Company and played an important part in the formation of the Salters' Institute of Industrial Chemistry. He served twice on the Council of the Royal Institute of Chemistry; he was a Vice-President of the Society in 1917–18.

Leach joined the Society in 1946. He was educated at John Ruskin School and Selhurst Grammar School and at Birkbeck College. After a period with British Drug Houses Ltd. he spent some years with the Printing and Allied Trades Research Association. From 1940 he was research chemist and analyst to British Waxed Wrappings Ltd. until 1944, when he moved to a similar position with T. Hubbuck & Son Ltd.

Mitchell died in his 90th year. He was educated at Longwood Grammar School, Huddersfield, and St. John's College, York, and received scientific training at Birkbeck College and the Royal College of Science, London. After holding the position of Science Master at Camberwell Grammar School he went to Tiverton Technical School as head of the Chemical Department. In 1895 he became Borough Analyst for Tiverton. He joined the Society in 1901.

Rudd Thompson became a Fellow of the Royal Institute of Chemistry in 1908 and had been a member of the Society since 1893. He died in his 80th year. He was President of the Society in 1924–5 during the Jubilee year, and he was the last President whose term of office was described in detail by Dr. Bernard Dyer in "*Fifty Years of the Society of Public Analysts*."

Trotman was 79 at the time of his death. He was Exhibitioner and Prizeman of St. John's College, Cambridge, and graduated as M.A. of the University of Cambridge in 1892. He became Science Master at Nottingham and in 1896 Public Analyst for the City of Nottingham, retiring from this post in 1937. He was Lecturer in Applied Chemistry at University College, Nottingham, from 1909 to 1938. He joined the Society in 1901.

Rowland Williams died in his 88th year. He assisted A. H. Allen in the preparation of Volume II of *Commercial Organic Analysis*. In 1885 he started a consulting practice in Manchester. He later became chief chemist of James Williamson & Son Ltd., of Lancaster, manufacturers of linoleum, leather cloth, etc. He became a member of the Society in 1887 and was elected a Fellow of the Institute of Chemistry in the same year. He was a Gas Identification Officer for Lunesdale during the recent war, in spite of his advanced age.

ORDINARY MEETINGS—Five meetings of the Society were held during the year and the following papers were communicated:—

"The Freezing Point of Bulk Milk." By F. J. Macdonald.
"The Determination of Cerium in Cast Iron." By W. Westwood, B.Sc., and A. Mayer.
"The Determination of Linoleic Acid in Edible Fats." By W. J. Stainsby, Ph.D., F.R.I.C.
"The Determination of p,p'—DDT in Commercial Samples." By A. E. Martin, B.Sc., F.R.I.C., and R. L. Wain, M.Sc., Ph.D., F.R.I.C.
"The Composition of Concentrated Tomato Puree and the Estimation of the Tomato Content of Tomato Ketchup." By J. C. Morpeth, B.Sc., A.R.I.C.
"Ether Peroxide as a Possible Source of Error in the Röse-Gottlieb Butter-fat Test." By M. M. Muers, Ph.D., F.R.I.C., and Miss M. A. House, B.Sc.
"The Standardisation of Hortvet Thermometers." By R. Aschaffenburg, Ph.D., and J. A. Hall, A.R.C.S., B.Sc., D.I.C.
"A Micro Method for the Determination of Unsaturation." By Miss W. M. Phillips and W. C. Wake, M.Sc., A.R.I.C.
"The Determination of Small Amounts of Hydroquinone in Styrene." By S. M. A. Whettem, B.Sc., F.R.I.C.
"A Method for Determining the Tin Content of Tungsten High Speed Tool Steel." By B. Bagshawe, A.Met., F.I.M., and E. Dyke, A.Met., A.I.M.

JOINT MEETING—The December Meeting was, as usual, a Joint Meeting with the Food Group of the Society of Chemical Industry. The subject was "Food Standards and Labelling," with the following special contributions:—

"Introduction." By C. A. Adams, C.B.E., B.Sc., F.R.I.C.
"The Viewpoint of the Manufacturer." By L. H. Lampitt, D.Sc., F.R.I.C., M.I.Chem.E.
"The Viewpoint of the Public Analyst." By H. E. Monk, B.Sc., F.R.I.C.

Mr. Monk, in making his contribution to the meeting, deputised at short notice for Mr. Stanley Dixon who was unable through indisposition to take part in the meeting.

NORTH OF ENGLAND SECTION—There have been five meetings during the year including a Joint Meeting with the Physical Methods Group and, for the first time since 1939, a Summer Meeting was held.

The following papers have been read and discussed:—

"The Government White Paper on the Post-War Loaf." By D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.

"The Examination and Analysis of Shellac." By A. Wright.
"Methods of Water Analysis." By W. Gordon Carey, F.R.I.C.
"Chemistry in the Kitchen." By E. B. Hughes, D.Sc., F.R.I.C.
"Analysis of Rare Earth Oxides by means of Emission Spectra." By D. M. Smith, B.Sc., D.I.C., F.Inst.P., and G. M. Wiggins.
"Determination of the Rare Earths, using the intermittent Arc." By J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.
"The Chromatographic Estimation of Vitamin A in Whale Liver Oil." By N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson and J. P. Savage, B.Sc., A.R.I.C.
"The Determination of the Meat Content of Sausages." By R. W. Sutton, B.Sc., F.R.I.C., and J. Markland, B.Sc., F.R.I.C.

SCOTTISH SECTION—In addition to the Annual General Meeting, two ordinary meetings ere held during the year.

One meeting in Edinburgh took the form of an Exhibition of Films. The subjects of the lms included the Cathode Ray Oscillograph, Crystals, Colour, Atomic Fission, Medicinal ases and Colloidal Chemistry. The show of films was followed by a Dinner at which the nembers of the Section joined with the members of the Association of Public Analysts of cotland.

The second meeting was held in Glasgow and the following paper was presented and liscussed:—

"The Determination of Vitamin B_1, Riboflavine and Nicotinic Acid by Microbiological and Chemical Methods." By James Cassidy.

The number of members of the Section is now 74, an increase of eight since last year.

MICROCHEMISTRY GROUP—Three meetings have been held during 1948, in London, berdeen and Leeds respectively. The Aberdeen meeting was held jointly with the Local ections of other Chemical Societies. The Leeds meeting was held with the Leeds Area ection of the Royal Institute of Chemistry and Leeds University Chemical Society.

The following papers have been read:—

"The Microchemical Aspects of Electrical Conductivity." By J. T. Stock, M.Sc., F.R.I.C.
"A New Micro Blowpipe for the Manipulation of Capillaries." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Diaphragm Pump for Air and Other Gases." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Melting Point Indicating Device." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Transmitting Manometer for Micro Oxygen Uptake Experiments." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"Ultra Micro Methods." By Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.
"Simultaneous Concentration of Trace Elements with Organic Precipitants." By R. L. Mitchell, B.Sc., Ph.D., A.R.I.C.
"Trace Determinations by Means of the Polarograph." By G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P.
"Micro Diffusion Analysis." By T. G. Brady.
"The Microscope as a Chemical Tool." By Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.
"General Account of Microchemical Methods in Forensic Investigations." By J. B. Firth, M.Sc., D.Sc., M.I.Chem.E., F.R.I.C.
"Microchemical Methods in Forensic Toxicology." By G. E. Turfitt.
"Rapid Colorimetric Methods for the Detection and Estimation of Alkaloids and Related Compounds." By E. Pedley.

The number of Group members is now 258, an increase of 48 since the last report.

The Committee has met three times during the year.

The University Grants Committee, having intimated that it was considering strengthening the teaching of microchemistry, invited the views of the Committee. A small Sub-Committee consisting of the Chairman, Professor Briscoe and Mr. Belcher prepared a memorandum on the subject which has been submitted to the U.G.C.

Steps were taken to form a Micro-analytical Methods Panel, to initiate investigations into microchemical methods, but the Committee found themselves unable to have a free hand in the organisation of such a panel and the matter was abandoned.

The report on "The Progress of Microchemistry in Germany" has now been published as "B.I.O.S. Final Report No. 1606" and is available to the general public.

Owing to pressure of work and also for health reasons the Hon. Secretary, Mr. Ronald Belcher, had to relinquish the office about July, and Mr. D. F. Phillips was appointed as Acting Hon. Secretary until the next Annual General Meeting.

PHYSICAL METHODS GROUP—During the past year the Physical Methods Group has held two meetings in London and one each in Birmingham, Leeds and Liverpool. One London meeting and the Liverpool meeting were held jointly with the Biological Methods Group and the North of England Section respectively. The Leeds meeting had been organised by the Polarographic Discussion Panel. The meetings had an average attendance of over 80 members and visitors. The following papers were read at meetings of the Group:—

Electron Microscope Meeting in London on November 25th, 1947.

"Electron Microscopy." By B. S. Cooper, B.Sc., F.Inst.P.

Penicillin Assay Meeting in London on January 29th, 1948.

"Introductory Survey of Physical and Chemical Methods." By E. Lester Smith, D.Sc., F.R.I.C.

"A Critical Review of some Proposed Methods for the Determination of Individual Penicillins." By W. R. Boon, B.Sc., Ph.D., F.R.I.C.

"The Determination of Penicillin by Alkaline Hydrolysis." By Stella J. Patterson, B.Sc., A.R.I.C., and W. B. Emery, B.Sc., A.R.I.C.

"The Spectroscopic Estimation of Penicillin." By G. H. Twigg, B.Sc., Ph.D.

"Introductory Survey of Biological Methods." By N. G. Heatley, M.A., Ph.D.

"Serial dilution Method" and "Differential Assay by Charcoal Adsorption." By C. G. Pope.

"The Microbiological Assay of Penicillin by the Turbidimetric Method using *Staphylococcus aureus*." By C. R. Bond, M.Sc.Tech., F.R.I.C., and O. L. Davies, M.Sc., Ph.D.

Tracer Isotope Meeting at Birmingham on April 2nd, 1948.

"Measurement of β-activity." By A. G. Maddock.

"Measurement of Radioactive Isotopes." By F. E. Whitmore.

"The Mass Spectrometer." By E. R. S. Winter, B.Sc., Ph.D., A.R.C.S., D.I.C., A.R.I.C

"Determination of Abundance Ratios of Non-radioactive Isotopes." By E. R. Roberts.

"Tracers in Biochemical Investigations." By W. J. Arrol.

Polaragraphic Meeting at Leeds on April 9th, 1948.

"Polarography in Germany." By G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P.

"The Polarographic analysis of Light Alloys and Metals." By W. Stross, M.D., F.R.I.C.

"The Polarography of Anions." By W. Furness, B.Sc., F.R.I.C.

Spectroscopy Meeting at Liverpool on October 2nd, 1948.

"Analysis of Rare Earth Oxides by means of Emission Spectra." By D. M. Smith, B.Sc., A.R.C.S., D.I.C., F.Inst.P., and G. M. Wiggins.

"Determination of the Rare Earths using the Intermittent Arc." By J. A. C. McClelland, B.Sc., Ph.D., F.R.I.C.

"The Chromatographic Estimation of Vitamin A in Whale Liver Oil." By N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson and J. P. Savage, B.Sc., A.R.I.C.

The Polarographic Discussion Panel held, in addition to the Leeds meeting, which was organised as a Group meeting, ordinary meetings at Norwood Technical College on December 12th, 1947, and at Imperial College on October 29th, 1948. The discussion at these meetings was opened by Mr. F. L. Steghart, Dr. F. L. Warren, Dr. S. G. Tudor Jones and Dr. J. E. Page. Mr. J. T. Stock, who has resigned from the office of Hon. Secretary of the Panel, must be thanked for the way in which he has organised the Panel during the last two years. Dr. W. Cule Davies is the Chairman of the Panel which now has 55 members.

The Group has been represented on the Barker Index Committee by Dr. J. G. A. Griffiths, Dr. J. H. Hamence and the Hon. Secretary.

The number of Group members is now 250, an increase of 67 since the last Annual Report.

BIOLOGICAL METHODS GROUP—The Group has held four meetings during the year. After the Annual General Meeting on December 16th, 1947, an ordinary meeting was held at which the following papers were read:—

"A Modified Method for the Microbiological Assay of Tryptophan, Methionine, Cystine and Tyrosine." By E. C. Barton-Wright, D.Sc., F.R.I.C., and N. S. Curtis.

"The Use of *Neurospora Crassa*, mutant 9185, for the Assay of Aneurine." By J. S. Harrison, B.Sc., M.Sc., and E. J. Miller.

"A Note on the Cup Method of Microbiological Assay and its Limitations." By W. F. J. Cuthbertson, B.Sc., Ph.D., F.R.I.C.

A Joint Meeting was held with the Physical Methods Group on January 29th, 1948, the subject being—

"Methods of Penicillin Assay—their Purpose, Scope and Validity."

Biological methods of assay were dealt with in papers by N. G. Heatley, M.A., Ph.D., C. G. Pope, and C. R. Bond, M.Sc.Tech., F.R.I.C., and O. L. Davies. Dr. A. A. Miles summed up at the end of a most vigorous and stimulating discussion. The meeting attracted many visitors, and undoubtedly served a most useful purpose in enabling the merits and applicability of physical and chemical methods to be assessed in relation to biological methods. The proceedings at the meeting appeared in full in *The Analyst* and have since been published separately in booklet form.

Dr. A. A. Miles, who is Head of the Department of Biological Standards at the National Institute for Medical Research, read a paper on Biological Standards at a meeting held on May 11th. A symposium was held on October 21st on—

"The Assay of Curare and Curarimimetic Substances."

Papers were read by C. A. Mogey, M.B., B.Ch., B.A.O., and J. W. Trevan, M.B., B.S., B.Sc., F.R.C.P.; G. B. West, Ph.D., B.Pharm.; J. Raventós, M.D.; H. O. J. Collier, B.A., Ph.D.; F. C. McIntosh, M.A., Ph.D.

The membership of the Group is now 135, 37 new members having been added during the year.

Dr. E. C. Wood, who has been Hon. Secretary of the Group since its inception, has resigned from this post, and Mr. S. A. Price has succeeded him.

PUBLIC ANALYSTS AND OFFICIAL AGRICULTURAL ANALYSTS COMMITTEE—The Committee met on three occasions during 1948. Amongst subjects discussed were how the Society could assist with mutual advantage the Consulting Pathological Group of the British Medical Association on the chemical side of pathological practice; examination of commercial petrol; and matters dealing with the welfare of Public Analysts in general.

ANALYTICAL METHODS COMMITTEE—The past year has shown the results of the considerable activities and progress of work of the Committee and its Sub-Committees. Five Reports from the Committee have been published during the year:—

The Evaluation of Powdered Tragacanth (*Analyst*, 1948, p. 368).
The Assay of Yohimba (*Analyst*, 1948, p. 309).
The Assay of Jaborandi (*Analyst*, 1948, p. 311).
The Assay of Ephedra and of Ephedrine in Nasal Sprays (*Analyst*, 1948, p. 312).
Determination of Traces of Zinc in Foodstuffs (*Analyst*, 1948, p. 304).

Sub-Committees have been active and progress reports indicate that a further report from the Tragacanth Sub-Committee is imminent and that the Standard Methods Sub-Committee has compiled a considerable bibliography. A Liaison Committee has been appointed to consider all cases in which the Society is invited to co-operate in the formulation of Standards and Standard Methods of Analysis. The Freezing Point of Milk Sub-Committee has been reconstituted and new Sub-Committees have been formed to investigate Standard Methods for Meat Extracts and for Soapless Detergents.

HON. TREASURER'S REPORT—The financial position of the Society continues to receive the closest attention. The Council set up, during the year, a small Sub-Committee to make a special study of the finances of the Society in view of the great increase in the cost of publication of *The Analyst* and of administrative expenses, and the preliminary recommendations of the Sub-Committee are now before the Council.

THE ANALYST—Some recovery from the effects of the fuel crisis of 1947 on the paper supplies for *The Analyst* is reflected in the increased size of the journal for 1948, which totals 704 pages, compared with 588 in 1947 and 600 in 1946. The numbers of original papers and notes published in 1948 were 78 and 33 respectively, compared with 61 and 19 in 1947, and the abstracts 470 compared with 304. In spite of this increased size the paper restriction to which the journal was subject led this year to a serious accumulation of papers and abstracts awaiting publication, and near the end of the year application was made for increased paper allocation. This has been granted and the immediate problem of how best to cope with the enlargement of the journal is receiving the attention of the Council.

Following the precedent of the cloth-bound reprints of Symposia on Polarography, Chromatography and Spectroscopic Analysis issued in 1946 (Annual Report, April, 1947), a similar reprint of the papers on "Methods of Penicillin Assay: their Purpose, Scope and Validity," held at a Joint Meeting of the Physical Methods Group and the Biological Methods Group, in January, 1948, has been published.

SPECIAL COMMITTEE ON TRAINING IN ANALYSIS—The Special Committee has drawn up a Memorandum during the year, which has been submitted to the Royal Society. It is hoped that the Memorandum will prove useful in drawing attention to the immediate need for much greater facilities for the training of students at Universities in Analytical Chemistry and will pave the way for prompt improvements.

SPECIAL COMMITTEE ON THE NAME OF THE SOCIETY, THE MEMORANDUM AND ARTICLES OF ASSOCIATION, AND RELEVANT MATTERS—The Special Committee appointed by the Council, to investigate these matters has obtained the opinion of the members on the subject of the title of the Society. The information thus gained has been carefully studied by the Council, and, as a result of their study, the Council expects to make recommendations to an Extraordinary General Meeting of the Society in the near future.

BIENNIAL LECTURE OF THE SOCIETY—Council has agreed unanimously that the Biennial Lecture given after alternate Annual General Meetings of the Society should be named: "The Bernard Dyer Memorial Lecture."

CHEMICAL COUNCIL—A grant of £500 has been made to the Society by the Chemical Council from funds accumulated during the war to assist in bringing the publication of original papers up to date.

INTERNATIONAL CONGRESS ON ANALYTICAL CHEMISTRY AT UTRECHT—The Society was well represented at this Congress, held last June.

PROPOSED INTERNATIONAL CONFERENCE ON ANALYTICAL CHEMISTRY—The success that attended the International Congress held at Utrecht has led to exploratory talks being held in London to investigate the possibility of holding a Conference in England in 1952. A General Committee, under the Chairmanship of Sir Robert Robinson, and an Executive Committee, with Mr. R. C. Chirnside as Honorary Secretary, were set up at a representative meeting held in the Rooms of the Royal Society to make preliminary arrangements for the Congress or Conference. The Society has made a grant for the preliminary expenses.

PLACE OF ORDINARY MEETINGS OF THE SOCIETY—For many years the Society has held its Ordinary Meetings in the Rooms of the Chemical Society in Burlington House, Piccadilly. The expansion of the Chemical Society has rendered it necessary for them to terminate this association at least temporarily. Ordinary Meetings of the Society have been held during the year at Gas Industry House, Hyde Park Corner, and at Manson House, Portland Place, London.

SEWAGE AND SEWAGE EFFLUENTS—The Society has appointed Dr. Hamence as representative on a technical committee of the Ministry of Health set up to consider necessary revisions in methods of chemical analysis in this field.

BRITISH STANDARDS INSTITUTION—Dr. G. W. Monier-Williams has been nominated as the Society's representative on the Chemical Divisional Council of the B.S.I. in place of Dr. E. B. Hughes, whose term of office has been completed.

BRITISH NATIONAL COMMITTEE ON CHEMISTRY OF THE ROYAL SOCIETY—Dr. A. M. Ward has been appointed the representative of the Society in place of Mr. F. W. F. Arnaud, whose term of office has been completed.

LEWIS EYNON, *President.*
K. A. WILLIAMS, *Hon. Secretary.*

Address of the Retiring President

LEWIS EYNON, B.Sc., F.R.I.C.

(*Delivered after the Annual General Meeting, March 9th,* 1949)

BEFORE dealing with the main subject of my address, I propose to make a short survey of the recent progress and activities of the Society.

The best criterion of the prosperity of a society is, I think, the rate of growth of membership. Taking the membership of our Society, in round numbers, at ten-year intervals for the last forty years: 350 in 1909, 450 in 1919, 600 in 1929, 850 in 1939 and 1500 in the present year, we are justified in feeling satisfied with the present and hopeful for the future. The membership has increased more than fourfold since 1909 and the increase has been greater in the past ten years than in the preceding thirty.

The strength of a society, however, depends not only on its numbers but on the spirit and activity of its individual members, and here again we have good reason for satisfaction. As compared with their London brethren, the country members of a society with its headquarters in London are at a considerable disadvantage, since for them attendance at meetings involves much expense and sacrifice of time. In 1925 members of the Society resident in the North of England met that difficulty by forming the North of England Section, with a Committee of Management, and in 1936 the Scottish members followed suit with the Scottish Section. The two Sections have flourished greatly and have added and do add to the strength and influence of the parent Society. I have been privileged to attend four Summer Meetings of the North of England Section and these gatherings remain as some of my pleasantest memories. A still more recent manifestation of the spirit and activity of our members has been the formation of three Groups for special subjects, called into existence by the rapid development of certain branches of analysis and covering this development very completely. I shall have occasion to refer to the Groups later.

Despite the upheavals of two World Wars and the consequent great increase in the cost of living, there is one commodity, and I can think of no other, the price of which remains the same as it was in 1914, and that is membership of the Society; we are legitimately proud of such a record. How has it been achieved?

Firstly, we owe a debt of gratitude to a succession of Honorary Treasurers whose careful husbandry has done so much to maintain our financial stability. Of our present Honorary Treasurer, Mr. G. Taylor, and of his immediate predecessor, Dr. E. B. Hughes, I can speak from personal experience, since I have served with them for years on the Finance Committee and on the Council, and know how jealously they have guarded the finances of the Society.

Secondly, we are indebted to a succession of Editors of *The Analyst*, under whose control our journal has not only acquired a very high prestige, but has become an asset of great value to the Society. Here again I can speak from personal experience of the work of our present Editor, Mr. J. H. Lane, and his immediate predecessor, the late Dr. C. A. Mitchell, since I have served with them for years on the Publication Committee. The editorial work has increased very much during the past few years, and this has necessitated the appointment of an Associate Editor, Mr. L. S. Theobald, and an Assistant Editor, Mr. F. L. Okell, and our thanks are due to them for the able assistance which they render to Mr. Lane.

I have one further observation to make with reference to our membership subscription. Although it is very gratifying that the subscription should have remained the same through the vicissitudes of the past thirty-five years, the welfare of the Society is our primary object and if, to ensure that, it became necessary to raise the subscription, I am sure that the Council would not hesitate to ask you to sanction that step.

I am glad to take this opportunity, the last I shall have, of thanking the Honorary Secretary, Dr. K. A. Williams, for his help to me during my term as President. Dr. Williams came new to his office two years ago and has carried out his duties as "to the manner born."

For some years past it has been our custom, in alternate years, to invite a distinguished chemist to give a lecture following the Annual General Meeting. The Council has decided that in future this biennial lecture shall be known as "The Bernard Dyer Memorial Lecture" in honour of the late Dr. Bernard Dyer who, through so many years, served the Society so well. It will be a most appropriate form of posthumous recognition, and will keep the memory of Dr. Dyer green long after those who knew him have passed away.

I now come to the main theme of my address—the fundamental importance of analysis to the progress of the science of chemistry, and the necessity of giving a prominent place to analysis in the training of the chemical student.

The same subject has been dealt with by two former Presidents of the Society—A. C. Chapman in 1915 and 1916[1] and E. R. Bolton in 1928[2]—and both deplored the inadequacy of training in analytical chemistry. The subsequent years, however, have shown little advance in the status of analysis as a subject of instruction in our universities and technical colleges; indeed, the position has, if anything, worsened owing to the increasing and admittedly irresistible claims of other branches of the science on the student's time. No apology, therefore, is needed for again pleading the claim of analytical chemistry to be regarded as the basis of the science and of prime importance in chemical education.

That this claim is fully justified is abundantly proved by the history of chemistry from the time of Lavoisier who, by quantitative experimental work on combustion, overthrew the Phlogiston Theory and transformed chemistry from an art into a science. The etymologist might reasonably object that Lavoisier's work on the conversion of elements into oxides is not analysis but synthesis; to the chemist, of course, the word "analysis" has so changed its meaning as to include its opposite.

A secondary effect of Lavoisier's work—hardly less important than the overthrow of the Phlogiston Theory—was the conviction that it gave to the then rising generation of chemists of the importance of exact quantitative work in furthering the progress of the science and the following period of sixty or seventy years saw an enormous development in the scope and accuracy of chemical analysis, a development which was of inestimable value both to theory and practice. It was during this period that methods of analysis were devised and perfected—and many of them by leaders of chemical thought—which are used to-day with little or no modification. It was during this period that the atomic weights of most of the elements were determined to a high degree of accuracy, the laborious work of many chemists on which the brilliant generalisation of the Periodic Law was built. It was during this period that Stas determined the atomic weights of a few of the elements, to a degree of accuracy that has not been surpassed, for the purpose of testing the validity of Prout's hypothesis that all elements are built up from hydrogen. The work of Stas led to the rejection of this hypothesis, and although more recent work on the nature of atomic structure has shown that the hypothesis has a basis of truth, it is, I think, quite certain that if the hypothesis had been accepted in its original, crude form, the development of atomic chemistry would have been hindered rather than helped. How many theories have been strangled at birth by the results of quantitative work? Most of us, I suppose, have found quantitative experiment to be a very salutary corrective of theoretical speculations.

Indeed, the first half of the nineteenth century might be called the Golden Age of analytical chemistry. As pointed out by Chapman it was followed by a period of comparative neglect which he justly ascribed to the enormous development of organic chemistry, a development to which the activity of research was chiefly directed. Chapman went on to prophesy that "just as modern organic chemistry was responsible for the neglect of the study of analytical chemistry, so the still more modern physical chemistry is likely to be responsible for its vigorous revival . . . and the same may be said of the many biological methods which are now being pressed into the service of analytical chemistry."

Chapman's prevision has been abundantly confirmed as is proved by the formation of the Physical Methods Group and the Biological Methods Group, and a new weapon has been added to the armoury of the analyst in the application of complex organic compounds as specific precipitants for various metals. Indeed, the rate of progress of analytical chemistry during the past thirty or forty years recalls the "Golden Age" of a century ago. The estimation of "trace" substances in various commodities has acquired an importance undreamt of previously. The metallurgist and engineer require accurate estimations of traces of foreign metals in the metals and alloys used in industry. The agricultural chemist requires accurate estimations of those traces of elements which have such remarkable effects on the fertility of soil, the quality of crops and the well-being of cattle. The food manufacturer requires accurate estimations of those "trace" substances—the vitamins—which are so necessary to health. The increasing stringency of Food Regulations requires accurate estimations of traces of substances injurious to health.

These requirements have been met by the development of physical and biological methods of analysis in recent years and, in addition, a new field of chemical analysis—microchemistry—of especial value when only very small samples of material are available, has been opened up. Traces of elements so small that they cannot be estimated by purely chemical methods or only by long and laborious processes, can be estimated by spectrographic and polarographic methods so rapidly that the results are available for the running control of factory processes. The possibilities of spectrographic methods have, of course, long been known, but their practical application in industry is of comparatively recent growth. It is only a few years since it was realised that traces of cobalt and boron are very important constituents of soil. It is safe to assume that in the future, other and perhaps more exacting demands will be made on the analyst, and that traces of substances not yet known to be of significance will prove to be so. More than that, substances now known to be poisonous when present even in traces may prove to be, not merely harmless, but actually beneficial when present in still smaller traces; indeed, fluorine is such a substance. There is another important branch of chemical analysis in which much more may be demanded from the chemist in the future, and perhaps in the near future, than in the past, *viz.*, the analysis of fertilisers and feeding stuffs. For many years it has been customary to make "omnibus" determinations of nitrogen and digestible carbohydrates without regard to the availability of these constituents. G. Taylor[8] has recently pointed out the inadequacy of such determinations for some types of fertilisers and feeding stuffs, and has foreshadowed the possibility of a new Fertilisers and Feeding Stuffs Act requiring the determination of "available" constituents.

In order that these increasing demands on the analyst shall be met it is necessary that the chemical student of to-day should be thoroughly trained in analysis.

Our Society was founded during the period of neglect of analytical chemistry and it has contributed greatly to the present or "Second Golden Age" by its meetings for the presentation and discussion of papers on analytical chemistry, by the work of its Analytical Methods Committee in devising new methods of analysis or modifying existing ones, by the publication of *The Analyst* and, within the last few years, by the formation of Groups for special subjects—the Microchemistry Group, the Physical Methods Group and the Biological Methods Group. The formation of these Groups is evidence, not only of rapid progress in but also of widened scope of analysis; it is evidence, too, that the individual chemist cannot hope to be a master in all branches of analysis.

It is fitting, therefore, that the Society should be concerned with the question of training the student of chemistry in analysis, since it is on this training that future progress in this branch of the science depends. One of the objects for which the Society was formed, as laid down in the Memorandum of Association, is "To encourage, assist and extend the knowledge and study of analytical chemistry."

None would question the great utilitarian value of training in analysis; the uses of analysis are too obvious and manifold. It serves as guide and control in manufacturing operations of the most diverse kinds, it is an indispensable aid to the medical man in the diagnosis and cure of disease and to the water engineer in ensuring supplies of wholesome drinking water, it is a protection to the public against the fraudulent trader and it is a Court of Appeal for the research worker in other branches of the science.

There does appear, however, to be an assumption by many, expressed or tacit, that training in analysis, though useful, is not of great educational value and that it should be subordinate to the study of the fundamental principles of chemistry. This assumption has no foundation in fact. Most of the fundamental principles of chemistry are derived from and based upon accurate quantitative work and the student learns in the most effective way possible, *i.e.*, from his own practice in quantitative analysis, the truth of those principles. As he becomes practised, beginning with the simplest determinations and progressing to the more difficult ones, he finds that after a time, depending on his aptitude, he can count on obtaining results within the commonly accepted limits of experimental error and that these limits can be narrowed but only by taking extraordinary precautions, and that there are limits which cannot be further narrowed. Thus, in learning to become a competent analyst the student not only acquires manipulative skill and dexterity, and familiarity with the use of various instruments of measurement; he learns for himself that chemistry is an exact science based on experimental work, and that the exact laws of chemistry, such as Avogadro's Law and the Law of Multiple Proportions, are reasonable deductions from experimental data, subject to experimental error. Such training must have the most salutary effect on the student's scientific mental outlook and critical faculty. He has been taught verbally the fundamental principles of chemistry; some of them at least he examines and verifies in the laboratory. His developed critical faculty leads him to investigate the causes of unexplained differences in quantitative results; it was the slight difference between the densities of "chemical" and "atmospheric" nitrogen that led Ramsay and Rayleigh to the discovery of argon, and the former to the discovery of the rarer gases of the atmosphere.

No one, I think, would suggest that the student should spend time on the study of special analytical methods devised for the special needs of industry; he could not hope to acquire the speed of those to whom such work is part of routine. If he has been thoroughly trained in the principles of analysis he will quickly pick up such specialised skill as and when required, and he will be competent to suggest improvements in special methods and to devise new ones.

What is now the status of analytical chemistry from the educational aspect in our teaching institutions? All the available evidence goes to show that it leaves much to be desired. A few years ago, under the auspices of the Committee of the Microchemistry Group, a questionnaire was addressed by Dr. C. L. Wilson to universities and technical institutions, and on the basis of the replies Dr. Wilson prepared a most admirable report entitled "The Teaching of Analytical Chemistry with Special Reference to Microchemistry,"[4] from which one conclusion may be quoted: "There is undoubtedly a general recognition among teachers of the importance of analytical chemistry in general, and microchemical methods have obviously recommended themselves strongly to teachers as a useful instructional topic. Recognition of this by the professional and other examining bodies is desirable, particularly in view of the probable post-war development. Every effort ought to be made to secure this recognition, and to make possible further development of the regard for analytical chemistry in teaching institutions."

Of the "professional and other examining bodies" referred to by Dr. Wilson, the Royal Institute of Chemistry fully recognises the importance of training in analysis. In 1940 the Institute added to its list of Branches of the Fellowship examination, Branch H, General Analytical Chemistry. The subject is an extremely wide one and, unlike some of the more specialised Branches, might be expected to attract a large proportion of candidates. Examination of the figures published in the *Proceedings* of the Institute, however, shows that the reverse is the case. During the years 1944–48, out of 188 candidates for the Fellowship examination (nine Branches) only 9 presented themselves for examination in Branch H. It seems that the only possible inference to be drawn from these figures is that candidates have not been sufficiently trained in analysis to satisfy the high standard set by the Institute. Anyone who regards analytical chemistry as a subject of subordinate importance in chemical education would do well to study the past examination papers of the Institute in Branch H; he should be effectively cured of that illusion.

In 1945 the then President of the Society, Dr. Monier-Williams, addressed a questionnaire to chemists occupying positions which would enable them to judge the competence in chemical analysis of recently qualified chemists. The majority of the replies were to the effect that the study of analysis did not receive sufficient time and attention during the years of studentship.

I think that the sum of the evidence from Dr. Wilson's report, from the figures of the Fellowship examination of the Royal Institute of Chemistry and from the replies to the questionnaire of Dr. Monier-Williams is amply sufficient to show that training in analysis does not occupy in the curricula of our teaching institutions the status that its importance warrants. To this it might be objected that progress in chemical analysis shows no sign of lessening, but the answer to that objection is that the effect of neglect of training does not of course become manifest for perhaps ten or twenty years. Once it does become manifest the cure is correspondingly slow, and the cure should obviously be begun as soon as possible.

What are the practicable steps in effecting the cure? Chapman[1] recommended the establishment of Chairs of Analytical Chemistry in our universities and colleges, a recommendation subsequently endorsed by Bolton[2] and again by Sir John Fox, then Government Chemist, at the 1937 Summer Meeting of the North of England Section. The adoption of this recommendation would undoubtedly give to analytical chemistry a status in our teaching institutions attainable in no other way, and some other countries, less industrialised than our own, have set the example. Further, Chairs of Analytical Chemistry would serve as foci of research in analysis which at present is too dependent on the unco-ordinated efforts of individual chemists generally made to meet an immediate need. More than the establishment of Chairs of analysis is required, however; as pointed out by Chapman and others a longer period of college training is necessary if chemical analysis is to be adequately taught, and this would certainly be welcomed by the teacher as well as by the student.

The chemical student of to-day has very much more to learn than was the case with his predecessor of fifty years ago. All branches of chemistry have developed greatly during this period and new branches, which must be studied, have come into being. Fifty years ago the student was taught to assume that the atom was a homogeneous, indestructible particle and that it was, so to speak, a "dead end" of chemistry. The development of atomic chemistry since then, and world events of the past four years have taught us that the atom is anything but a "dead end" and to-day the study of the structure of the atom and of the nature of valency is of the utmost importance in the student's training; no one would suggest that less time should be given to it. The enlargement of our chemical vocabulary too has kept pace with the development of the science, and this entails a further tax on memory.

These increased demands on the student can only be met by a longer period of training, either by lengthening the ordinary course or by the provision of post-graduate courses. Unless one of these alternatives is adopted and if the student continues to be offered more mental food than he can assimilate in the time now at his disposal, he will either suffer from mental indigestion or, what perhaps is more probable, he will, consciously or unconsciously, reject the excess. In either case the progress of chemistry as a whole, and of chemical analysis in particular, will suffer.

There is no body in this country so closely concerned with the question of training in analysis as our Society. Two years ago the Council appointed a Special Committee to consider the question and make recommendations with a view to improving the present state of affairs. The Committee prepared a Report, subsequently approved by the Council, and this Report has been submitted to the appropriate authorities.

If the present unsatisfactory conditions of training in chemical analysis are allowed to continue there is serious danger that within, say ten or twenty years, the analyst himself may be "weighed in the balance and found wanting."

REFERENCES

1. *Analyst*, 1915, **40**, 77; 1916, **41**, 31.
2. *Ibid.*, 1928, **53**, 190.
3. *Thirtieth Streatfeild Memorial Lecture*, 1947 (Royal Institute of Chemistry publication).
4. *Nature*, 1945, **156**, 588.

The Micro-Determination of Potassium as Cobaltinitrite in Biological and Agricultural Materials

Part II. A New Turbidimetric Method

BY J. TINSLEY

THE development of turbidimetric methods of analysis for a wide variety of purposes was described up to 1929 by Yoe.[1] In agricultural science such methods have been applied chiefly to studies on soil fertility and plant nutrition. Much attention has been paid to potassium and the various procedures used for the turbidimetric determination of this element in soil extracts have been reviewed by Tinsley.[2] For the routine examination of extracts obtained with a solution of sodium acetate and acetic acid prepared according to Morgan,[3] the method devised by Tinsley and Pizer[4] for use with the Spekker absorptiometer, has proved very useful. In this method a stock solution of sodium cobaltinitrite was prepared according to Bennett[5] and freshly diluted 1 in 5 with Morgan's solution before use. For each series of tests 0·5 ml. of diluted sodium cobaltinitrite reagent was mixed with 1 ml. of soil extract in a small glass tube and the potassium cobaltinitrite cloud precipitated with 1 ml. of a cold mixture of methyl and *iso*propyl alcohols. This latter mixture was added as a layer at 0° C. and then mixed in a standard manner by means of a mechanical reciprocating shaker. Interference by ammonium ions was effectively prevented by adding 1 drop of a 1 per cent. solution of sodium hypochlorite to the soil extract before the addition of the sodium cobaltinitrite. Turbidity was measured directly in the cylindrical test tube mounted in a brass holder on the "Spekker." The only serious defect of the method was the interference caused by appreciable amounts of sulphate present in some extracts.

It was considered that if this interference by sulphate could be overcome and the procedure for cloud formation simplified, with improved stability of the cloud, the turbidimetric determination might be applied generally to other solutions containing potassium. Bechold and Hebler[6] prepared stable dispersions of barium sulphate in glycerol and Baver and Bruner[7] advocated the use of glycerol to stabilise the oxalate precipitate obtained in their turbidimetric test for calcium. The author has investigated the possibility, using glycols either in place of or combined with the usual alcohols, to form the potassium cobaltinitrite cloud.

EXPERIMENTAL

PRELIMINARY WORK—

It was found that Bennett's sodium cobaltinitrite solution could be mixed with alcohol - glycol mixtures to give reagents that produced a cobaltinitrite precipitate suitable for turbidimetric measurement when added to various solutions containing small quantities of potassium. Cloud formation was investigated with various mixtures of methyl, ethyl (95 per cent.) or *iso*propyl alcohol with glycerol, ethylene glycol, or propylene glycol (1 : 2 dihydroxypropane).

Reagents—(i) Morgan's acetate solution containing 10 g. of AnalaR hydrated sodium acetate and 3 ml. of AnalaR glacial acetic acid per 100 ml.

(ii) Standard solutions of potassium chloride dissolved in Morgan's solution, containing from 5 to 50 p.p.m. of potassium.

(iii) Similar standard solutions containing in addition to potassium known amounts of purified gum arabic, calcium acetate, magnesium acetate, sodium phosphate and sodium sulphate, respectively.

(iv) Standard potassium solutions in water alone, and in solutions containing respectively 22 per cent. of sodium perchlorate, 0·5 N acetic acid, 0·5 N calcium acetate, 1 per cent. of citric acid, an equivalent amount of calcium citrate, and 4 per cent. of trichloracetic acid.

(v) Sodium cobaltinitrite solution. Initially Bennett's solution was used containing 5 g. of hydrated cobalt nitrate, 30 g. of sodium nitrite and 2·5 ml. of acetic acid per 100 ml. This was replaced after trial by a solution containing 20 g. of AnalaR sodium cobaltinitrite and 20 g. of AnalaR sodium nitrite per 100 ml. which proved more satisfactory for cloud formation, easier to prepare and more stable when stored.

(vi) Alcohol - glycol mixtures. Each alcohol was re-distilled and mixed in different proportions with each glycol.

(vii) Reagent mixtures of the sodium cobaltinitrite solution (v) with the alcohol - glycol mixtures were freshly prepared before use at the same temperature as was used for cloud formation, because of the slow decomposition of the cobaltinitrite. The solubility of the sodium cobaltinitrite in the reagent mixture decreased as the proportion of alcohol was increased. The order of solubility in the presence of alcohols was in methyl > in ethyl > in *iso*propyl; as to glycols, the solubility in presence of glycerol was about the same as in presence of ethylene glycol and much greater than in presence of propylene glycol. With glycerol especially there was a strong tendency to supersaturation, but only those reagent mixtures that were true stable solutions proved satisfactory for cloud formation.

Procedures for cloud formation and measurement—The potassium solution and reagent mixture were mixed in five different proportions by volume, which are distinguished as procedures (*a*), (*b*), (*c*), (*d*) and (*e*). Usually a final volume of 3 ml. of test liquid was obtained, and for this the following volumes were required.

Procedure	Reagent mixture ml.	Potassium solution ml.
(*a*)	1·00	2·00
(*b*)	1·20	1·80
(*c*)	1·50	1·50
(*d*)	1·80	1·20
(*e*)	2·00	1·00

(i) *Micro procedure* using the test tubes directly for turbidity measurements on 3 ml. of final mixture.

Small round-bottomed glass tubes* 70 mm. long by 14 mm. outside diameter were used, as previously employed by Tinsley and Pizer. The reagent mixture was added in two ways, either by running it carefully down the side of the tube to form a layer above or below the potassium solution depending on its density, or by rapid delivery from a pipette with a large orifice to achieve mixing as quickly as possible. In each case mixing was completed immediately with a glass plunger made from a piece of rod by forming a flattened disc at one end about 10 mm. in diameter so that it could be moved up and down in the tube. The tubes were held in a metal tray which could be immersed in an ice-bath or water-bath to secure a controlled temperature. Turbidity measurements were made on the "Spekker" with the aid of a brass tube holder and with the No. 1 red glass filters and a drum setting for the blank test of 1·50 or 1·00 depending on the range required.

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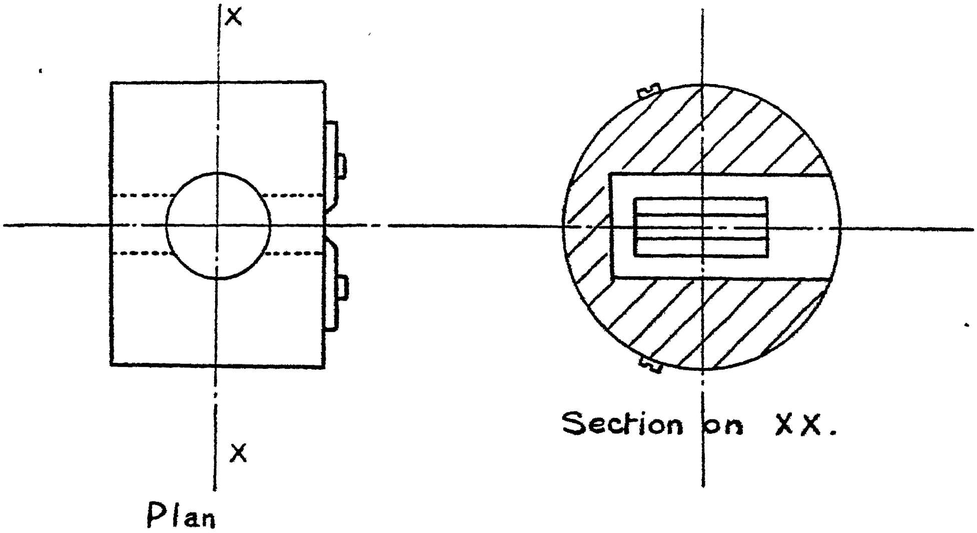
* Supplied by Wood, Bros. Glass Co., Barnsley.

Fig. 1—Design of Brass Test Tube Holder



The design of the tube holder is shown in Fig. 1. It was made from a piece of brass cylinder of diameter 38 mm. (1·5 inch). A hole just slightly exceeding 14 mm. in diameter was bored 30 mm. deep in a central position between the two plane parallel faces of the cylinder which had an over-all length of 30 mm. when finished. A rectangular window was cut from each face to the central hole; these two windows were centrally placed, parallel, and measured 8 mm. by 18 mm. The glass test tube when in position in the hole and containing the test liquid, formed a cylindrical lens of focal length approximately 14 mm. Two semi-circular plates were fixed to one face of the holder by means of threaded screws to form a slit of variable width behind the window. The slit was finally adjusted to an optimum width of 3 mm. which, when facing the photo-cell, cut off most of the dispersed light, yet allowed sufficient to pass for adequate sensitivity on the ordinary Spekker model. A small screw on either side of the holder ensured that when it was placed on the sliding cell carrier of the instrument the test tube was always in a vertical position.

(ii) *Macro procedure* using standard 1-cm. rectangular cells for measurement.

By using hard glass test tubes 18 mm. in diameter the same procedure for cloud formation could be followed but with four times the volumes, giving a final volume of test liquid of 12 ml. This amount was quite adequate to fill the 1-cm. cells usually employed with the "Spekker," and thus enabled slightly greater accuracy of measurement to be attained.

Results—These have been recorded in detail by Tinsley[8] and only the more important points arising from the preliminary experiments are mentioned here. The five procedures were compared at 0° and 15° C., with two levels of Bennett's cobaltinitrite solution, namely, 0·15 ml. and 0·30 ml. per 3 ml. of the final mixture. With all the reagent mixtures examined procedures (*c*) and (*d*) gave the best turbidity. The differences between turbidities at the two temperatures and the two levels of cobaltinitrite were irregular but not very pronounced. Turbidity increased with the concentration of alcohol in the reagent mixture up to the limit of solubility of the sodium cobaltinitrite, this effect being most evident with the lower potassium standards and reagent mixtures containing glycerol and ethylene glycol. Propylene glycol gave good turbidity without any alcohol. Methyl alcohol gave the most satisfactory turbidity with each glycol and the best results were secured with mixtures of methyl alcohol and propylene glycol.

Two variations of the original method of Tinsley and Pizer were tried. Firstly, with each glycol incorporated in the diluted sodium cobaltinitrite solution and mixed with the potassium solutions before addition of the alcohols separately. Secondly, with the sodium cobaltinitrite solution added to the potassium solutions before the addition of the alcohol-glycol mixtures separately. Neither procedure proved as successful as the use of the combined reagents containing sodium cobaltinitrite, methyl alcohol and propylene glycol.

CLOUD FORMATION WITH REAGENT MIXTURES CONTAINING METHYL ALCOHOL, PROPYLENE GLYCOL AND GLYCEROL—

Good turbidity was secured with mixtures containing methyl alcohol and propylene glycol with a smaller proportion of glycerol or ethylene glycol. The presence of either of the last two enabled a higher proportion of alcohol to be present in the reagent mixture than with propylene glycol alone. Turbidity tests favoured glycerol rather than ethylene glycol, and in this series of experiments different proportions of these three organic reagents were used in combination with 10 volumes per cent. of sodium cobaltinitrite solution (20 per cent. + 20 per cent. of sodium nitrite). Procedure (*c*) was used at 15° C., with standards in Morgan's solution and in water, the reagent mixture being added as a layer before mixing except in tests on the duplicated standards, and for these the cloud was formed in one tube by the "layering" procedure, and in the other tube by rapid mixing. Readings were taken 20 and 40 minutes after mixing but only the former are given here; they are recorded as drum readings × 100 in Table I.

TABLE I

CLOUD TURBIDITY WITH REAGENT MIXTURES CONTAINING METHYL ALCOHOL, PROPYLENE GLYCOL AND GLYCEROL

Mixture		P.p.m. potassium in acetate solution								P.p.m. K in water	
No.	M.P.G.	5 B	5	10	20	30	40	50	50 B	50	50 B
1	7.1.1	130	129	110	87	67	65	45	56	26	54
2	7.0.2	135	134	114	76	53	51	16	31	4	20
3	6.2.1	133	132	116	96	73	66	47	60	27	10
4	6.1.2	133	133	113	74	42	22	1	0	4	0
5	6.0.3	139	140	116	80	46	24	4	9	11	5
6	5.3.1	135	135	114	77	45	23	4	4	16	4
7	5.2.2	135	136	115	79	45	26	11	11	34	8
8	5.1.3	145	145	117	81	52	27	17	31	9	↓ 16
9 ↓	5.0.4	147	147	121	82	49	32	7	23	11	18
10	4.4.1	136	136	114	77	45	23	1	2	10	↓ 8
11	4.3.2	138	136	114	79	46	29	6	24	9	↓ 10
12 ↓	4.2.3	146	146	124	88	51	26	8	10	10	10
13 ↓	4.1.4	150	150	124	86	51	27	13	13	12	16
14 ↓	4.0.5	150	150	128	88	52	30	13	13	13	13
15	3.6.0	134	134	114	79	47	26	5	5	10	↓ 9
16	3.5.1	137	138	118	78	48	27	5	4	22	↓ 8
17 ↓	3.4.2	143	142	119	86	54	25	4	7	14	13
18 ↓	3.3.3	144	146	120	84	45	27	5	5	12	8
19	2.7.0	138	137	119	78	47	24	3	5	33	↓ 7
20 ↓	2.6.1	140	138	124	80	48	26	3	4	22	5
21 ↓	2.5.2	144	145	118	80	47	28	6	14	14	9
22 ↓	1.8.0	137	137	118	80	49	30	25	7	16	16
23 ↓	1.7.1	142	141	120	81	48	34	11	12	11	19
24 ↓	0.9.0	137	140	118	80	44	26	4	8	10	11

M = Methyl alcohol, volumes per 10 volumes of reagent mixture.
P = Propylene glycol, volumes per 10 volumes of reagent mixture.
G = Glycerol, volumes per 10 volumes of reagent mixture.
B = Reagent blown into potassium solution.
↓ = Reagent sunk *below* potassium solution.

With reagent mixtures 1 to 3, containing 60 to 70 per cent. of methyl alcohol, layering was not good because diffusion occurred at the interface, and turbidity was poor with the higher standards both in acetate and water solution. For the rest it was noticed that as the glycerol concentration increased, visible cloud formation was delayed. With reagent mixtures 12 to 14, containing 30 to 50 per cent. of glycerol, this delay amounted to about 5 seconds with the higher standards, but with the lowest standard cloud formation was very slow and the turbidity increased for 4 hours after mixing. Thus glycerol retards cloud formation, possibly by restricting molecular movement within the solution. Because mixing could be completed before the cloud formed appreciably, reproducibility of the turbidity was good and also the stability of cloud was good, but the slow rate of formation with the lower standards would be a serious disadvantage when testing solutions covering a wide range of potassium content.

Rapid mixing was generally as good as the layering procedure with the acetate solutions; and with the water solutions rapid mixing gave greater turbidity with the 50 p.p.m. standard. This was encouraging, because if the reagents proved satisfactory in other respects mixing could be done in a simple standard manner. Reagent mixtures Nos. 6, 10 and 15 appeared to give the best gradation of turbidity with adequate sensitivity for the 5 p.p.m. solution. These gave closely similar results when tested under a wide variety of conditions, but No. 6 appeared to be slightly superior over-all, and in the following account only results for this reagent are given.

CLOUD FORMATION WITH REAGENT MIXTURES PREPARED FROM 50 VOLUMES OF METHYL ALCOHOL, 30 VOLUMES OF PROPYLENE GLYCOL AND 10 VOLUMES OF GLYCEROL PER 100 VOLUMES—

(*i*) *Effect of sodium cobaltinitrite concentration*—Reagent mixtures containing 7·5, 10 and 12·5 volumes per cent. of sodium cobaltinitrite solution were freshly prepared and used at 15° C. by procedure (*c*). The results recorded in Table II show that the turbidity of the lower standards decreased as the concentration of sodium cobaltinitrite fell below 10 per cent., but was not significantly increased by raising the concentration above this level. The reagent mixture containing 12·5 per cent. proved to be supersaturated and a precipitate of sodium cobaltinitrite was deposited after use.

TABLE II

EFFECT OF SODIUM COBALTINITRITE CONCENTRATION ON TURBIDITY

Cobaltinitrite solution % of reagent mixture	Time, min.	P.p.m. K in acetate solution						P.p.m. K in water
		5	10	20	30	40	50	50
7·5	20	140	119	84	54	25	6	13
	40	138	120	86	56	29	10	16
10	20	132	113	82	49	27	14	16
	40	132	112	84	52	30	17	20
12·5	20	132	111	76	45	23	1	8
	40	129	109	75	47	24	6	10

(*ii*) *Effect of alcohol - glycol concentration*—Reagent mixtures containing 12·5, 10 and 8·3 volumes per cent. of sodium cobaltinitrite solution were prepared and used at 15° C. by procedures (*b*), (*c*) and (*d*) respectively, thus giving 35, 45 and 55 volumes per cent. of alcohol - glycol mixture in the final state after cloud formation with 1 per cent. each of sodium cobaltinitrite and sodium nitrite throughout. The results recorded in Table III show that

TABLE III

EFFECT OF ALCOHOL - GLYCOL CONCENTRATION ON TURBIDITY

Procedure used	Time, min.	P.p.m. K in acetate solution						P.p.m. K in water
		5	10	20	30	40	50	50
(*b*)	20	140	117	74	35	7	0	6
	40	140	114	73	36	12	0	8
(*c*)	20	136	115	79	47	23	5	7
	40	135	113	80	49	25	9	12
(*d*)	20	137	124	94	65	40	22	47
	40	137	123	94	65	42	25	49

the turbidity, as measured, was least for the lowest standards and greatest for the highest standards by procedure (*b*). However, when the readings were plotted against actual concentrations of potassium in the final mixture it was evident that procedure (*b*) gave a lower turbidity throughout. Procedures (*c*) and (*d*) gave almost identical turbidities, showing that the maximum effect of the alcohol - glycol mixture was obtained in each case.

(*iii*) *Effect of temperature*—It was decided to compare the turbidity produced at 10°, 15° and 20° C. by procedures (*c*) and (*d*), using the same reagent mixture throughout. The results recorded in Table IV show that with both procedures the turbidity decreased with rise of temperature, the difference between 10° and 15° C. being smaller than between 15° and 20° C. The decrease in turbidity was relatively most marked with the lowest potassium concentration as would be expected. 15° C. seemed to be well justified as the most convenient temperature for ease of laboratory control combined with good turbidity.

TABLE IV

EFFECT OF TEMPERATURE ON TURBIDITY

		P.p.m. potassium in acetate solution											
		Procedure (*c*)						Procedure (*d*)					
Temp.	Time, min.	5	10	20	30	40	50	5	10	20	30	40	50
10° C.	20	134	116	78	45	21	0	131	116	89	61	38	20
	40	133	115	78	47	24	5	131	116	88	61	38	20
15° C.	20	136	115	80	46	23	4	134	119	92	64	39	21
	40	135	115	79	49	26	8	134	119	92	66	41	24
20° C.	20	141	118	83	53	28	11	137	122	94	68	46	25
	40	139	116	84	55	32	16	137	122	94	68	45	27

(*iv*) *Effect of calcium, magnesium, phosphate and sulphate*—Using the same standard solutions as in the preliminary experiments and reagent mixture of the same composition as in the previous experiment, clouds were formed at 15° C. by procedures (*c*) and (*d*). The turbidity readings were taken 30 minutes after mixing. The results recorded in Table V show that none of these elements present in the amounts stated, either separately or all together, has any serious effect on the turbidity.

TABLE V

EFFECT OF CALCIUM, MAGNESIUM, PHOSPHATE AND SULPHATE AND ALSO AMMONIUM CHLORIDE AND SODIUM HYPOCHLORITE ON TURBIDITY

	Procedure (*c*)						Procedure (*d*)					
P.p.m. K in	5	10	20	30	40	50	5	10	20	30	40	50
Acetate solution	135	118	80	50	25	5	132	118	89	62	44	24
+ 5000 p.p.m. Ca	135	118	80	51	25	1	132	119	86	64	40	26
+ 1000 p.p.m. Mg	137	120	86	54	26	6	132	118	87	64	45	27
+ 100 p.p.m. P	137	116	86	55	27	6	133	118	88	64	45	26
+ 1000 p.p.m. SO_4	135	117	85	51	25	6	132	119	88	64	43	27
+ Ca + Mg + P + SO_4	136	117	83	54	29	6	132	118	86	62	45	26
+ 1 drop 10% NaOCl solution							132	117	90	63	44	25
+ 250 p.p.m. NH_3 + 1 drop NaOCl solution							130	116	86	62	44	23

(*v*) *Effect of ammonium chloride and sodium hypochlorite*—In these experiments 1 drop of B.D.H. 10 per cent. sodium hypochlorite solution was added to each tube containing 1·2 ml. of standard potassium solution in two sets, one containing the usual range of potassium standards in Morgan's acetate solution, and the other containing also 250 p.p.m. of ammonia (NH_3) added as ammonium chloride. Turbidity readings taken 30 minutes after mixing and recorded in Table V show that the ammonium ion was completely immobilised by the sodium hypochlorite solution, which had no effect by itself.

(*vi*) *Method of mixing for cloud formation and accuracy of the results*—Duplicate tests were made with procedures (*c*) and (*d*), at 15° C., in one case the reagent mixture being layered and in the other blown in rapidly from the pipette before completing the mixing with a glass plunger. Each set of tests was replicated ten times on different occasions and the readings were made 30 minutes after mixing. The results are summarised in Table VI showing the mean "Spekker" reading for each standard solution, 150 minus the mean, the standard deviation and the coefficient of variation. Similar information is included for the previous method of Tinsley and Pizer.

TABLE VI

COMPARISON OF METHODS OF CLOUD FORMATION

	P.p.m. K in acetate solution					
	5	10	20	30	40	50
Procedure (*c*) layered:						
Mean reading × 100	134·9	115·1	80·4	50·8	26·2	7·4
150 − Mean	15·1	34·9	69·6	99·2	123·8	142·6
Standard deviation	1·20	1·60	1·62	2·55	2·17	2·72
Coefficient of variation	7·9	4·6	2·3	2·6	1·7	1·9
Rapid mixing:						
Mean reading × 100	135·9	115·8	81·1	49·8	25·9	6·4
150 − Mean	14·1	34·2	68·9	100·2	124·1	143·6
Standard deviation	1·00	1·84	2·24	1·81	1·45	2·35
Coefficient of variation	7·0	5·4	3·2	1·8	1·2	1·7
Procedure (*d*) layered:						
Mean reading × 100	134·9	119·8	91·5	65·5	43·6	23·7
150 − Mean	15·1	30·2	58·5	84·5	106·4	126·3
Standard deviation	1·52	2·20	3·22	2·20	1·74	2·67
Coefficient of variation	10·1	7·3	5·5	2·6	1·7	2·1
Rapid mixing:						
Mean reading × 100	134·7	119·5	90·4	64·4	42·0	22·2
150 − Mean	15·3	30·5	59·6	85·6	108·0	127·0
Standard deviation	1·64	2·17	2·35	1·62	1·33	1·51
Coefficient of variation	10·7	7·2	3·9	1·9	1·2	1·2
Method of Tinsley and Pizer:						
Mean reading × 100	138·5	121·4	93·1	70·8	51·8	35·8
150 − Mean	11·5	28·6	56·9	79·2	98·2	114·2
Standard deviation	1·96	1·31	1·66	0·92	1·84	1·40
Coefficient of variation	17·0	4·6	2·8	1·2	1·5	1·2

Comparison of the coefficients of variation indicates that procedure (*c*) gave slightly better reproducibility than procedure (*d*) and there was little difference between layering and rapid mixing with either procedure. Rapid mixing gave slightly greater turbidity with the higher standards by procedure (*d*). The new reagent mixture gives better turbidity than the old method using methyl and *iso*propyl alcohols with the 5 p.p.m. standard, but otherwise there is little difference, apart from the facts that the new reagent mixture does not show any effect of sulphate present in solution, and the "life" of the cloud is longer.

CLOUD FORMATION WITH SOLUTIONS OF POTASSIUM CHLORIDE IN WATER, 0·5 *N* ACETIC ACID, 4 PER CENT. TRICHLOROACETIC ACID AND 22 PER CENT. SODIUM PERCHLORATE SOLUTION—

Sodium perchlorate solution was included because of its extensive use by Bray[9] for extracting the exchangeable potassium from soils, but a sample of the salt obtained from a

TABLE VII

TURBIDITY WITH SOLUTIONS OF POTASSIUM CHLORIDE IN WATER, 0·5 *N* ACETIC ACID, 4 PER CENT. TRICHLOROACETIC ACID AND 22 PER CENT. SODIUM PERCHLORATE SOLUTION

		P.p.m. potassium											
		Procedure (*c*)						Procedure (*d*)					
Solution in	Time, min.	5	10	20	30	40	50	5	10	20	30	40	50
Water	20	143	120	85	54	30	19	137	123	94	71	48	21
	40	141	120	84	55	32	22	135	123	94	71	50	26
0·5 *N* acetic acid	20	145	120	84	54	29	8	137	122	94	66	44	22
	40	143	120	84	55	32	12	135	122	94	66	47	25
4% CCl_3COOH	20	150	150	100	66	32	13	137	123	91	64	42	21
	40	150	150	96	65	35	17	135	123	91	64	45	24
22% $NaClO_4$ solution	20	142	118	80	52	30	8	137	120	89	64	41	21
	40	140	114	80	54	32	10	135	120	90	64	45	25

chemical supplier contained an appreciable quantity of potassium and it was necessary to prepare a solution free from potassium; this was prepared from A.R. perchloric acid and sodium bicarbonate. Clouds were formed by procedures (*c*) and (*d*) at 15° C. and readings were taken 20 and 40 minutes after mixing. The results recorded in Table VII show that the turbidity obtained with water, acetic acid and sodium perchlorate solutions was closely similar to that with Morgan's acetate solution, particularly with the latter two solutions. With trichloroacetic acid the readings for the lower standards showed very low turbidity by procedure (*c*) but were almost normal by procedure (*d*). This was thought to be due to the greater buffering capacity of the larger quantity of reagent employed in the latter and this point was examined more fully later. Procedure (*d*) gave slightly improved turbidity with the lowest standard for each solution and the calibration curves when plotted were more nearly linear.

METHOD RECOMMENDED FOR GENERAL USE

Procedure (*d*) appeared from the experimental work to be most suitable for the turbidimetric determination of potassium in various solutions containing from 5 to 50 p.p.m. The following method was adopted for routine use.

REAGENTS—

(i) *Potassium standards* prepared in the appropriate solution for calibration of the "Spekker" and routine checking of the method, especially when a new batch of reagents is prepared. It is convenient to maintain a stock solution containing 1·907 g. of AnalaR potassium chloride per litre, corresponding to 1000 p.p.m. of K, from which the working standards of 5, 10, 20, 30, 40 and 50 p.p.m. are prepared by dilution as required.

(ii) *Sodium hypochlorite solution.* A commercial 10 per cent. stock solution should be stored in a refrigerator if possible and freshly diluted to 1 per cent. with water each day as required.

(iii) *Alcohol - glycol mixture.* This can be prepared and stored in bulk by mixing 5 volumes of methyl alcohol with 3 volumes of propylene glycol and 1 volume of glycerol. Re-distillation of these reagents is not usually necessary provided they are quite free from suspended material.

(iv) *Sodium cobaltinitrite solution* containing 20 g. of sodium cobaltinitrite and 20 g. of sodium nitrite per 100 ml. The special precautions necessary for its preparation and storage are discussed below.

(v) *Sodium cobaltinitrite - alcohol - glycol reagent mixture.* This is freshly prepared before use by mixing 1 volume of (iv) with 9 volumes of (iii). The special precautions necessary are also discussed below.

APPARATUS—

Small test tubes for micro-tests, 70 mm. long by 14 mm. in diameter, selected for uniformity. Pyrex test tubes for macro-tests, 150 mm. long by 18 mm. in diameter. Metal racks of convenient size for holding tubes when immersed in the water-bath. Thermostat bath with a stirrer and well lagged, to maintain 15° C. Glass rods of two sizes approximately 80 mm. and 170 mm. long with flattened discs at one end approximately 10 mm. and 14 mm. in diameter respectively; one rod is used for each test tube. Pipettes 1 ml., 2 ml., 5 ml. and 10 ml. straight form, Grade B, those used for the reagent mixture having a large orifice and graduated to the tip.

Spekker photo-electric absorptiometer or other suitable instrument. Brass test tube holder as described previously (p. 169) for use with the "Spekker."

PROCEDURE—

As many as 20 to 30 tests may be carried out in one batch. When interference by ammonium ions must be eliminated it is convenient to add the necessary drops of 1 per cent. sodium hypochlorite solution to the test tubes prior to the potassium solution so that mixing is automatically secured. The tubes containing the test solution are held in the appropriate rack and immersed to a convenient depth in the water-bath adjusted to 15° C. by adding ice or warm water when necessary. The reagent mixture, prepared as described below, is also immersed in the bath for 5 to 10 minutes before use. Then the required volume is delivered as a jet of liquid into the potassium solution at a rate of approximately 1 ml. per second from a suitably calibrated pipette having a large orifice and held in a vertical position in the tube. [illegible] the outside of the pipette becomes contaminated by splashing with the test liquid or in other ways it must be wiped clean since it is most important not to introduce crystal nuclei into the bulk of the reagent mixture.

Volumes for	Micro-tests	Macro-tests
[illegible] per cent. sodium hypochlorite	1 drop	4 drops
Potassium solution	1 ml. or 1·2 ml.	4 ml.
Reagent mixture	1·5 ml. or 1·8 ml.	6 ml.
Total volume	2·5 ml. or 3·0 ml.	10 ml.

A blank test on the reagents without potassium is included in each batch in order to set the "Spekker" with a drum reading of 1·50 or 1·00, using the red light filters. It is also advisable to include a standard potassium solution with each batch as a check on the calibration curve.

The tubes are removed from the water-bath 15 minutes (or 30 if desired) after mixing was started and the turbidity measurements are made in the same order so that each tube is always read at about the same time after mixing. For the micro-tests each tube is carefully wiped clean and dry on the outside and then inserted in the brass tube holder mounted on the cell carrier close to the slider. A final volume of 2·5 ml. in the tube may not be sufficient to cover the full depth of the window of the holder, in which event the larger volumes must be used to give 3 ml. of test liquid. For macro-tests the contents of the tube are poured into the 1-cm. glass cell in the usual manner.

PREPARATION AND PROPERTIES OF STOCK SODIUM COBALTINITRITE SOLUTION AND REAGENT MIXTURES—

Experience has shown[*] that careful attention to detail is necessary in the preparation of the reagent mixture if reliable results are to be secured from day to day. During some measurements on aqueous solutions it was observed that the turbidity was frequently considerably below normal, especially when the freshly prepared reagent was first used; after some time it generally improved. This was thought to be due to the formation of crystal nuclei of sodium cobaltinitrite in the reagent mixture. These may arise either in the stock sodium cobaltinitrite solution or be formed when it is added to the alcohol - glycol mixture. The following recommendations are made for the preparation of sodium cobaltinitrite solution and reagent mixtures.

Dissolve the required amount of sodium cobaltinitrite in water. Add the same amount of sodium nitrite crystals, stir until dissolved and dilute to the required volume to give 20 per cent. w/v of each constituent. Store for three or four days, preferably in a refrigerator and then filter. This may be done with a No. 44 Whatman filter paper, or by means of a sintered glass filter funnel of the finest porosity, but neither method appears to remove crystal nuclei completely. The best results are secured by using a sintered glass funnel of small Buchner type, having a disc of coarse porosity impregnated with an acetic acid - collodion membrane. This may be prepared quite simply by pouring on the 2 per cent. solution of collodion in glacial acetic acid to form a thin layer of liquid, allowing it to soak into the pores and then filling the funnel with distilled water before applying suction. A steady rate of filtration should be maintained and if the membrane is kept immersed in water when not in use it may be stored for repeated use. With filtration under suction it is important that the cobaltinitrite solution be collected in a large boiling tube inside the flask, or in some other manner so that it does not drip from the stem of the funnel and splash the walls of the receiving vessel. If exposed in thin films under reduced pressure evaporation of water will cause precipitation of crystal nuclei, and for this reason it is important that filtration be sufficiently rapid for this loss by evaporation to be negligible. It is a good plan to measure the volume of the filtrate, and then if necessary dilute to the right concentration The stock solution should be stored in a dark glass bottle preferably with a wide mouth and with a well-fitting screw cap. It may be stored for a year in the refrigerator without deterioration, and at room temperature for at least a month. Care should be taken when removing aliquots with a pipette to prevent liquid drying on the walls or neck of the flask and so contaminating the bulk with crystal nuclei.

It is convenient to measure the required 9 volumes of alcohol - glycol mixture into a large boiling tube or other suitable container that can be immersed in the water-bath at

15° C. The stock sodium cobaltinitrite solution, if stored in the refrigerator, should be warmed to room temperature or 15° C. before adding the necessary 1 volume to 9 volumes of the alcohol - glycol mixture. The stem of the pipette used for this purpose should be carefully wiped with a piece of clean filter paper to remove any drops of cobaltinitrite solution from the outside before running the contents directly into the alcohol - glycol mixture, so that no films of cobaltinitrite liquid are exposed to evaporation and deposition of crystal nuclei. Mixing should be completed with a glass plunger having a bulb blown at one end. A rise in temperature occurs on mixing and the reagent mixture should be allowed to remain suspended in the bath for 5 or 10 minutes before use.

The reagent mixture is not stable for more than 2 hours at 15° C., but this is sufficient time for conducting a large number of tests. It should not be exposed to direct sunlight.

SOME APPLICATIONS OF THE METHOD

(*a*) *Cloud formation in solutions of Morgan's acetate mixture, 0·5 N acetic acid, 0·5 N calcium acetate, 1 per cent. citric acid and its equivalent of calcium citrate, water and 22 per cent. sodium perchlorate solution*—Fig. 2 shows that the turbidity was closely similar throughout.

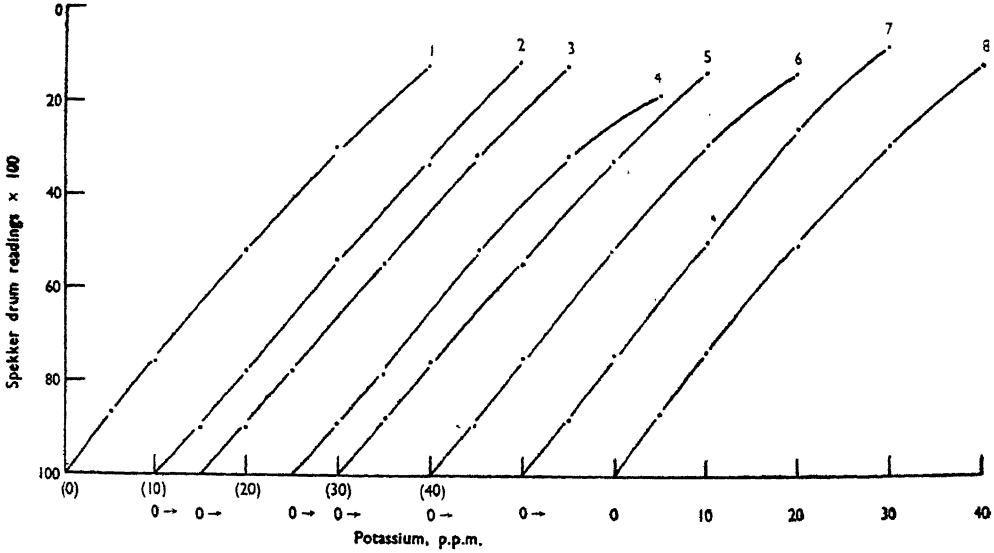


Fig. 2. Turbidity curves for potassium standards in various solutions.

1. Morgan's acetate solution.
2. 0·5 *N* Acetic acid.
3. 0·5 *N* Ca acetate.
4. 1% Citric acid.
5. Ca citrate solution
6. Water.
7. NaClO$_4$ solution.
8. CCl$_3$COOH solution.

The potassium graduations for all these curves are similar but only those for curves 1 and 8 are set out in full, the former in brackets.

Other tests showed that sodium hypochlorite was effective for removing ammonium in each case. These results suggested that potassium may readily be determined turbidimetrically in soil extracts prepared with these solutions.

(*b*) *Determination of potassium in soil extracts*—(i) *With Morgan's acetate solution*—Ten soil samples of differing texture, and contents of organic matter, calcium carbonate and exchangeable potassium were extracted with solution in the ratio 1 : 2. The potassium was precipitated from 100 ml. of each extract by adding 10 g. of sodium cobaltinitrite and storing in the refrigerator overnight. The precipitate was then filtered on to a Gooch crucible packed with asbestos, washed with 0·01 *N* nitric acid and re-dissolved in hot 5 *N* nitric acid and the solution evaporated to dryness. The potassium was re-precipitated and determined by the volumetric cobaltinitrite method of Wilcox.[10] 4-Ml. portions of each soil extract were also measured in duplicate into test tubes for the turbidimetric determination of the potassium by the macro-procedure. The results recorded in Table VIII show that the mean difference between turbidimetric results and those by the Wilcox method was —0·65 per cent. of the latter with a standard deviation of 2·21 per cent. The greatest difference was —7·6 per cent. obtained with the smallest amount of potassium, and although this might have been better, the results are reasonably good on the whole, for the direct turbidimetric determination of potassium in soil extracts.

TABLE VIII

DETERMINATION OF POTASSIUM IN SOIL EXTRACTS

Soil extract	Method of Wilcox p.p.m.	Turbidimetric method p.p.m.	Difference p.p.m.	Difference % of Wilcox figure
	(i) In Morgan's Acetate Extracts			
1	34·8	33·3	− 1·5	− 4·3
2	41·9	39·5	− 1·4	− 3·3
3	9·2	8·5	− 0·7	− 7·6
4	14·5	15·0	+ 0·5	+ 3·4
5	18·9	19·0	+ 0·1	+ 0·6
6	51·7	51·0	− 0·7	− 1·3
7	29·4	29·5	+ 0·1	+ 0·3
8	17·6	18·5	+ 0·9	+ 5·1
9	28·0	29·0	+ 1·0	+ 3·6
10	26·3	25·5	− 0·8	− 3·0
	(ii) In 1 per cent. Citric Acid Extracts			
11	7·1	7·8	+ 0·7	+ 9·9
12	4·8	5·0	+ 0·2	+ 4·2

(ii) *With 1 per cent. citric acid solution*—Two soils, one acid and one calcareous were extracted with 1 per cent. citric acid in the usual manner and the potassium was determined turbidimetrically directly on the extract and also by the normal analytical method of Wilcox after extracting the ash. The results recorded in Table VIII show that agreement is fair and, though not numerous enough to warrant definite conclusions, they suggest that a direct turbidimetric determination in citric acid extracts of soils may prove sufficiently accurate for much routine advisory work.

(*c*) *Cloud formation in solutions of trichloroacetic acid*—A 4 per cent. solution of trichloroacetic acid is commonly used for the precipitation of proteins in blood serum, and milk. Many different methods have been used for the determination of potassium in the filtrates, and a reliable turbidimetric method would prove very useful. In earlier experiments procedure (*c*) did not give full precipitation of the potassium, and it was confirmed by the macro-method that procedure (*d*) was satisfactory. The solution has an initial pH of 0·8, which is very acid, but no advantage was gained by buffering it at pH 5 to 6 with sodium acetate solution before adding the reagent mixture. In the normal way the blood serum is diluted five-fold to give a concentration of potassium in the filtrate ranging between 35 and 60 p.p.m., and the milk is diluted ten-fold to give a concentration of potassium ranging between 150 and 200 p.p.m. Therefore it is generally necessary to dilute the blood and

TABLE IX

TURBIDITY WITH SOLUTIONS OF POTASSIUM IN 4 PER CENT. TRICHLOROACETIC ACID

(i) With standard solutions

	P.p.m. potassium				
Macro-procedure (*d*):	5	10	20	30	40
Mean reading × 100	86·6	73·6	50·8	29·8	11·8
100 − Mean	13·4	26·4	49·2	70·2	88·2
Standard deviation	1·93	1·74	2·13	2·31	2·61
Coefficient of variation	14·4	6·6	4·3	3·3	2·9

(ii) Determination of potassium in blood and milk filtrates

Filtrate	Method of Wilcox p.p.m.	Turbidimetric method p.p.m.	Difference p.p.m.	Difference %
Blood	60·2	60·0	+ 0·2	+ 0·3
Milk	195·0	190·0	− 5·0	− 2·6

milk filtrates further before a turbidimetric determination can be made, and this dilution can be done with water.

Readings were obtained with a series of potassium standards in 4 per cent. trichloroacetic acid replicated sixteen times on different occasions. The mean values together with the statistical information are given in Table IX. The calibration curve plotted from these values is included in Fig. 2.

(*d*) *Determination of potassium in blood serum and milk*—A sample of ox blood was obtained and the serum separated. The proteins were precipitated from 200 ml. to give a total volume of 1 litre. In the same way the proteins were precipitated from 100 ml. of separated milk, with the aid of heating to 70° C., to give a total volume of 1 litre. Duplicate 200-ml. samples of blood filtrate, and duplicate 50-ml. samples of milk filtrate were evaporated in silica dishes. In the final stages the residues were moistened with a few ml. of concentrated sulphuric acid and further evaporated on sand-baths before completing the ignition in a muffle furnace. The potassium was extracted from the residues with hot water and determined by the volumetric method of Wilcox. Aliquots of the original filtrates were diluted 1 in 2 and 1 in 10 respectively, and the clouds were formed in duplicate tubes by the macro-turbidimetric procedure. Readings were made on the "Spekker," 30 minutes after mixing, and the potassium content read off from the calibration curve. The results recorded in Table IX show a good agreement between the standard analytical method and the rapid turbidimetric determination.

SUMMARY

Aqueous sodium cobaltinitrite solution may be used together with an alcohol and a glycol in a single reagent mixture for the precipitation of potassium cobaltinitrite as a finely divided cloud suitable for turbidimetric determination. The most convenient temperature for precipitation is 15° C. The reagent mixture that appeared to give best results contained 10 volumes of sodium cobaltinitrite solution (20 per cent. + 20 per cent. of sodium nitrite), 50 volumes of methyl alcohol, 30 volumes of propylene glycol and 10 volumes of glycerol, but a fair variation in the relative amounts of alcohol and glycol is possible without affecting the turbidity seriously, provided the true limit of solubility of sodium cobaltinitrite is not exceeded. For reliable results crystal nuclei must be absent from the reagent mixture and potassium solutions.

The cloud may be formed in a small volume of 2·5 to 3 ml. in a cylindrical glass tube and the turbidity measured directly with the aid of a test tube holder designed for use on the "Spekker"; or the precipitation may be done in larger volume in a test tube and the reading made in a rectangular glass cell.

The turbidimetric method gave good quantitative estimations of potassium in various soil extracting solutions and also in trichloroacetic acid filtrates from blood and milk. Compared with the volumetric cobaltinitrite method of Wilcox the results normally did not differ by more than 5 per cent.

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DEPARTMENT OF AGRICULTURAL CHEMISTRY
THE UNIVERSITY, READING

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The Colorimetric Estimation of Thiols

BY F. N. WOODWARD

IN the course of another investigation the need arose for a method for the rapid estimation of small quantities of

(I) 2-hydroxyethanethiol, monothioethyleneglycol, $HO.CH_2.CH_2.SH$

in solution in

(II) 2 : 2′-dihydroxydiethyl sulphide, thiodiglycol, $(HO.CH_2.CH_2)_2S$.

The iodine titration method of Klason and Carlson,[1] the mercuric chloride method of Sampey and Reid[2] and the copper oleate method of Bond[3] were all found to be capable of giving accurate results, although none was applicable to the analysis of small quantities of the thiol - sulphide mixture. Efforts were therefore made to develop a colorimetric micromethod.

Tasker and Jones,[4] Lecher and Seifken[5] and Rheinboldt[6] have demonstrated that primary, secondary and tertiary thiols react with nitrosyl chloride to form coloured thionitrites, *viz.*—

$$R.SH + NOCl = R.SNO + HCl.$$

Later, Rheinboldt[7] showed that ethyl mercaptan, thiophenol and triphenyl thiocarbinol react similarly with nitrous acid in aqueous alcohol solution; he claimed that the colours develop gradually on standing and are stable in concentrated but evanescent in dilute solution.

It has been found that the cherry red 2-hydroxyethane-1-thionitrite, $HO.CH_2.CH_2.SNO$ obtained in alcoholic solution by interaction between (I) and aqueous sodium nitrite in presence of glacial acetic acid is rapidly developed and is perfectly stable for at least 10 minutes both in dilute and concentrated solution, and that the coloured solution obeys Beer's law over a wide range (0·5 to 5 mg. per ml.) and consequently is ideal for the purposes of quantitative colorimetric estimation.

The Hilger Spekker photo-electric absorptiometer was used in the development of the method, and the 1-cm. cuvette (10-ml. capacity) and No. 5 filter were found to combine maximum sensitivity with maximum range.

DEVELOPMENT OF COLOUR—

The best method for making the coloured solutions required for the standard curve was found to be as follows: x-ml. of a solution of the pure thiol (I) in absolute alcohol (containing 0·02516 g. per ml.) were pipetted into the cuvette by means of a graduated pipette; $(6-x)$ ml. of absolute alcohol were then added from a burette, followed by exactly 1 ml. of 4 N aqueous sodium nitrite and finally exactly 2 ml. of glacial acetic acid A.R., making a total of 9 ml.

The contents of the cuvette were then mixed immediately by shaking, and readings taken on the absorptiometer after 2, 3, 5, 8, 11 and 15 minutes. The results obtained are recorded in Table I.

TABLE I

Filter: 5. Cuvette: 10 mm. Range of curve: 5 to 150 mg. of (I)

Volume of thiol (I) solution taken ml.	Wt. of thiol (I) g.	Absorptiometer readings after 2 min.	3 min.	5 min.	8 min.	11 min.	15 min.
0·2	0·0050	0·055	0·057	0·057	0·057	0·056	0·051
0·5	0·0126	0·121	0·125	0·124			
1·0	0·0252	0·245	0·261	0·267	0·267	0·267	0·267
1·5	0·0378	0·342	0·369	0·369	0·369	0·369	0·366
2·0	0·0503	0·371	0·440	0·450	0·460	0·465	0·467
3·0	0·0755	0·480	0·560	0·575	0·595	0·600	0·605
4·0	0·1006	0·568	0·645	0·675	0·699	0·703	0·710
5·0	0·1258	0·658	0·710	0·738	0·770	0·775	0·778
6·0	0·1509	0·665	0·740	0·780	0·790	0·805	0·815

From these results it is apparent that the more dilute solutions rapidly reach the point of maximum colour intensity, and maintain their maximum colour for about 5 minutes only before beginning to fade; with increase in concentration, both the time taken to reach maximum intensity and the stability increase. Provided the timing is made accurately it is possible to get exactly reproducible results, and for the purposes of the proposed method the standard curve is made from the 3-minute readings; only the reading after 3 minutes is required when carrying out the analysis.

It is essential that the sodium nitrite solution used should be exactly 4 *N*, as any variation in the strength directly affects the intensity of the coloured solution. A 4 *N* solution has been found to be the best in practice, as more dilute solutions give less intense colours, whilst from more concentrated solutions solid sodium nitrite tends to be precipitated on the addition of alcohol, and even if this does not occur the final increase in colour intensity is very small compared with that obtained from a 4 *N* solution, although rapidity of colour development increases with increase in concentration of the sodium nitrite solution. This is shown by the findings recorded in Table II, where the only variable is the concentration of the aqueous sodium nitrite solution used.

TABLE II

Filter: 5. Cuvette: 10 mm. (I) in 6 ml. of EtOH + 1 ml. of aq. $NaNO_2$ + 2 ml. of AcOH

Concn. of aq. $NaNO_2$ used	Wt. of thiol (I) in 9 ml.	Absorptiometer readings after							
	g.	2 min.	5 min.	8 min.	11 min.	15 min.	18 min.	22 min.	30 min.
2 *N*	0·0693	0·273	0·320	0·340	0·350	0·364	0·370	0·387	0·410
4 *N*	,,	0·550	0·590	0·618	0·618	0·620	0·620	0·620	0·620
6 *N*	,,	0·610	0·630	0·630	0·630	0·630			

TABLE III

Percentage of (I) in mixture of (I) and (II)	Wt. of mixture per 50 ml. of Solution A	Absorptiometer readings	Wt. of (I) per 5 ml. of Solution A, found from standard curve	Percentage of (I) in mixture, found
	g.		g.	
0	10·5420	0·0; 0·0	0·0	0·0
3·98	10·2110	0·415; 0·410	0·0440	4·31
6·48	6·2885	0·398; 0·402	0·0420	6·68
10·1	4·1129	0·398; 0·400	0·0420	10·2
15·6	2·6113	0·400; 0·400	0·0420	16·0
49·8	0·8174	0·395; 0·388	0·0410	50·2
100	0·4058	0·392	0·0405	100

TABLE IV

Filter: 5. Cuvette: 10 mm.

Percentage of (I) in mixture of (I) and (II) as made up	Weight of (I) in 9 ml. of solution used in cuvette	Absorptiometer readings		Percentage increase in absorptiometer reading due to presence of (II) 100(A−B)/B
		A With (II)	B Without (II)	
	g.			
1·02	0·0209	0·235	0·222	5·9
1·02	0·0419	0·426	0·401	6·2
2·01	0·0417	0·409	0·386	6·0
3·23	0·0207	0·225	0·213	5·6
3·30	0·0417	0·408	0·386	5·7
4·02	0·0417	0·406	0·386	5·2
5·18	0·0417	0·402	0·386	4·2
5·86	0·0209	0·228	0·222	2·7
5·86	0·0419	0·412	0·401	2·7
7·21	0·0209	0·223	0·222	0·4
7·21	0·0419	0·406	0·401	1·2
8·16	0·0419	0·406	0·401	1·2

EFFECT OF EXCESS OF THIODIGLYCOL—

The efficacy of the method as a means of estimating (I) quantitatively in the presence of (II) was assessed by analysing mixtures of (I) and (II) in various known proportions. An amount of the thiol - sulphide mixture containing about 0·4 g. of the thiol (I) was accurately weighed, and made up to 50 ml. with absolute alcohol (solution A). Five ml. of this solution thus obtained, containing approximately 0·04 g. of (I), were then examined by the colorimetric method. The results are shown in Table III.

From these results it is apparent that the presence of the sulphide (II) tends to enhance the colour intensity, but this increase never represents an error of more than 0·4 unit in the percentage of (I) in the mixture, and as an error on the amount of (I) present it only becomes serious when the concentration of (I) in the mixture is less than about 6 per cent. Even in these circumstances, however, the method is applicable, as the percentage increase in colour intensity is constant for a given thiol - sulphide ratio irrespective of the dilution of the mixture in the cuvette. These percentage increases in colour intensity were determined experimentally, with the following results.

Neither 2 : 2′-dihydroxyethyl sulphide (II), chloroethylchlorovinyl sulphide, 2 : 2′-di(β-chloroethylthio)diethylether nor dithian gives coloured solutions with nitrous acid.

The method finally developed for the estimation of (I) in an inert diluent or admixed with (II) is as follows.

METHOD

Reagents required—Glacial acetic acid A.R., absolute alcohol, and aqueous sodium nitrite solution, exactly 4 *N*.

Procedure—Weigh accurately an amount of the material to be examined containing not more than 0·4 g. and not less than 0·1 g. of monothio-ethyleneglycol into a 50-ml. graduated flask and dilute with absolute alcohol to exactly 50 ml.

Transfer 6 ml. of this solution by means of a graduated pipette into a Hilger 10-mm. (10-ml. capacity) cuvette and add exactly 1 ml. of the 4 *N* aqueous sodium nitrite solution from a burette, followed immediately by exactly 2 ml. of glacial acetic acid A.R. from another burette. Start a stop-watch at once and intimately mix the contents of the cuvette by shaking. Measure the intensity of the coloured solution on the Spekker photo-electric absorptiometer exactly 3 minutes after the addition of the acetic acid.

Use No. 5 green filters on both sides of the lamp.

Standard solution—It is necessary to check the standard curve from time to time, and for this purpose a standard solution is prepared containing exactly 0·5 g. of pure monothio-ethyleneglycol per 100 ml. of alcohol solution, and 6 ml. of this are treated exactly as described above. A standard solution thus prepared will keep for several weeks in the dark in a stoppered bottle filled to the neck, and can be checked by iodine titration.

Calculation—

If the weight of sample originally taken = w g.
If the reading of abscissa on the gauging curve corresponding to absorptiometer reading of the unknown = a
and that of the standard = b
Then the percentage of monothioethyleneglycol in the sample

$$= \frac{0{\cdot}03 \times a \times 50 \times 100}{6 \times w \times b} = 25a/bw \qquad .. \quad .. \quad .. \quad .. \qquad \text{(i)}$$

The result thus obtained is accurate in the examination of monothioethyleneglycol alone or in presence of thiodiglycol in concentrations greater than 6·5 per cent. If the monothioethyleneglycol content thus found is less than 6·5 per cent. then the percentage of monothioethyleneglycol = $25af/bw$, where f is a correction factor obtained from the following table, based on the results shown in Table IV, corresponding to the approximate monothioethyleneglycol content found by (i).

Percentage of (I) found by equation (i)	Correction factor f
0 to 4·0	0·945
4·0 to 5·0	0·954
5·0 to 5·5	0·962
5·5 to 6·0	0·973
6·0 to 6·5	0·985

APPLICATIONS—

The method appears to be of general applicability to thiols. Standard curves for 2 : 2′-dimercaptodiethyl ether, $O(C_2H_4SH)_2$, ethyl mercaptan, C_2H_5SH, and 2-hydroxy-propane-1-thiol, HO.CHMe.CH$_2$.SH, were made in addition to that for 2-hydroxyethane-1-thiol. The data obtained for them are recorded in Table V.

TABLE V

DATA FOR STANDARD CURVES FOR ETHYL MERCAPTAN, 2-HYDROXYETHANE-1-THIOL, 2-HYDROXYPROPANE-1-THIOL AND 2 : 2′-DIMERCAPTODIETHYL ETHER

Filter: 5. Cuvette: 10 mm.

Normal quantities of EtOH, 4 *N* $NaNO_2$ and acetic acid

C_2H_5SH		$HO.C_2H_4.SH$*		$HO.CHMe.CH_2.SH$		$O(C_2H_4SH)_2$	
g.	absorption	g.	absorption	g.	absorption	g.	absorption
0·01829	0·225	0·01	0·130	0·0118	0·108	0·0354	0·382
0·03658	0·390	0·02	0·243	0·0236	0·227	0·0708	0·588
0·05487	0·515	0·03	0·345	0·0354	0·335	0·1062	0·708
0·07316	0·598	0·04	0·424	0·0472	0·410		
0·09145	0·655	0·05	0·492	0·0590	0·495		
		0·06	0·529	0·0708	0·522		
		0·07	0·580	0·0826	0·565		
		0·08	0·613	0·0944	0·580		
		0·10	0·668	0·1180	0·650		
		0·12	0·700	0·1416	0·685		

* These absorptiometer readings differ from those recorded in Table I on account of differences between the galvanometers used.

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H.M. RESEARCH ESTABLISHMENT
SUTTON OAK

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The Polarographic Estimation of Inorganic Nitrates in Propellants

BY J. PEARSON AND A. J. HOWARD

POTASSIUM nitrate is sometimes incorporated in propellants and has been estimated by a polarographic method, which depends upon a determination of potassium in the ashed propellant and calculation to nitrate. The potassium content found must be corrected for the amount of sodium also present. An estimation of nitrate, which is specific to the added ingredient, would be much sounder in principle.

EXPERIMENTAL

The method employed for the estimation of nitrate was that proposed by Kolthoff, Harris and Matsuyama,[1] and a calibration graph relating the diffusion current to nitrate concentration was prepared by the method to be described further on.

To each of six 250-ml. beakers was added 0·3 g. of nitroguanidine and 0, 2, 4, 6, 8 and 10 mg. of potassium nitrate in aqueous solution. The contents of the beakers were made up to approximately 80 ml. and heated just to boiling to bring all the nitroguanidine into solution. They were then allowed to cool to room temperature, whereupon most of the nitroguanidine crystallised out. They were filtered through No. 41 papers into 100-ml. measuring flasks and washed with water so that the volume of filtrate and washings was 100 ml. Twenty ml. of each solution were pipetted into glass evaporating basins and 4 drops of methyl orange indicator and 4 ml. of *N* aqueous potassium hydroxide were added. The solutions were evaporated to dryness on a steam-bath and the residues washed into 50-ml. measuring flasks with about 20 ml. of water. These solutions were then titrated with *N* hydrochloric acid to the first permanent pink coloration, 10 ml. of the supporting electrolyte were added and the volumes made up to the mark. Portions of the resulting solutions were then polarographed, and when the diffusion currents were plotted against the amount of nitrate the points fell on the calibration graph previously prepared. This indicated that the separated crystalline nitroguanidine had not adsorbed any measurable amount of nitrate and that no nitrate was produced or lost during the alkaline destruction of the dissolved nitroguanidine.

Nitrocellulose is not reducible at the dropping mercury cathode, but when decomposition of nitroguanidine has to be effected it is of importance that nitrocellulose should not gain access to the solutions to be so treated, as it is decomposed by alkali to give compounds such as nitrates and nitrites which would interfere with the estimation desired. In the determination of nitrate in normal propellants the nitrocellulose is purposely precipitated in a very fine form to facilitate removal of inorganic salts, a condition which favours the colloidal suspension of nitrocellulose. Fortunately, this precipitation is not necessary when dealing with propellants containing nitroguanidine in relatively large proportions. These have a more "open" structure and water-extraction removes all soluble material leaving the nitrocellulose behind in a coarse form. To test whether any interference would arise from suspended nitrocellulose the following experiment was carried out. A sample of a nitroguanidine propellant, which contained potassium sulphate and no nitrate, was submitted to the procedure described below under Method. No nitrate was found. To a similar sample, potassium nitrate equivalent to 1·2 per cent. was added. On carrying out an estimation the same proportion was found. This indicates that in spite of the use of rapid filter papers (No. 41) the amount of nitrocellulose that gained access to the aqueous extract was, for all practical purposes, negligible.

METHOD

REAGENTS—

Supporting electrolyte—This contains 0·5 M potassium chloride, 0·05 M hydrochloric acid and 0·001 M uranyl acetate.

Potassium hydroxide—Normal solution.

Hydrochloric acid—Normal solution.

CALIBRATION—

Prepare an aqueous solution containing 0·200 g. of pure dry potassium nitrate per litre. Place 0, 2, 4, 6, 8 and 10 ml. of this in six 50-ml. measuring flasks. Add exactly 10 ml. of the supporting electrolyte to each flask and make up to the mark with water. Mix well and polarograph a portion, after flushing out with nitrogen or hydrogen, at appropriate galvanometer sensitivity, between —0·75 and —1·5 volts *versus* the internal mercury anode. Measure the diffusion currents and plot against the amount of added nitrate.

ESTIMATION OF POTASSIUM NITRATE IN PROPELLANTS CONTAINING NITROGUANIDINE—

Extract 1·000 g. of the ground propellant with carbon tetrachloride for 6 hours in a Soxhlet apparatus, to remove nitroglycerine, etc. Dry the residue at 100° to 105° C. and transfer it as completely as possible to a dry 150-ml. beaker. Add about 50 ml. of boiling water and stir vigorously. Decant the liquid through a No. 41 Whatman paper into a 250-ml. conical flask, retaining the nitrocellulose in the beaker. Continue the washing by decantation with hot water until a total volume of about 150 ml. has been used. Wash the thimble in which the propellant had been extracted with about 30 ml. of boiling water, adding the washings to the conical flask. Allow the liquid to cool to room temperature and to crystallise. Filter through a No. 41 Whatman paper into a 200-ml. measuring flask, wash the residue with water and make up the volume of the filtrate and washings to the mark. Mix well, pipette 20 ml. of the solution into a glass evaporating basin, add a few drops of methyl orange indicator and 4 ml. of N potassium hydroxide, and evaporate to dryness on a steam-bath. Wash the residue with about 20 ml. of water into a 50-ml. measuring flask and add N hydrochloric acid slowly from a burette until a permanent pink coloration just appears. Add exactly 10 ml. of the supporting electrolyte, make up to the mark with water and mix well. Polarograph a portion of the solution as described above. Measure the diffusion current and calculate the content of potassium nitrate by reference to the calibration graph.

ESTIMATION OF POTASSIUM OR OTHER INORGANIC NITRATE IN PROPELLANTS NOT CONTAINING NITROGUANIDINE—

Extract 1·000 g. of the ground propellant with ether (sp.gr. 0·720) for 6 hours in a Soxhlet apparatus and remove most of the ether from the residue by drying in an oven. Transfer the nitrocellulose to a 150-ml. conical flask and dissolve it in 20 to 25 ml. of acetone. Add, slowly and with constant agitation, 70 ml. of water to precipitate the nitrocellulose in a finely divided form. Heat cautiously, with frequent swirling, on a steam-bath until all the acetone is evaporated and then dry completely. Add 100 ml. of water from a pipette, stopper the flask and shake it vigorously to dissolve the inorganic salts. Filter through a dry No. 42 Whatman paper, rejecting the first runnings. Alternatively the nitrocellulose may be washed and the complete water extract made up to 100 ml. To a 25-ml. measuring flask add 5 ml. of the supporting electrolyte and a measured volume of the filtered water extract. Make up to the mark with water, mix, polarograph as described above, and calculate the nitrate content. Since there is a limit to the concentration of nitrate, beyond which the diffusion current is no longer proportional to the concentration,[1] if the diffusion current is greater than can be read from the calibration graph take another polarogram on a solution made up with a smaller volume of the water extract. If the diffusion current is too small to be conveniently measured, use a correspondingly larger volume of the water extract.

RESULTS

Some typical results of the estimation of potassium nitrate in propellants are given in Tables I and II.

TABLE I

PROPELLANTS CONTAINING NITROGUANIDINE

Propellant	KNO_3 found polarographically %	KNO_3 calculated from potassium content determined as $KClO_4$ %
1	1·20; 1·16	1·16; 1·17
2	1·14; 1·16	1·17; 1·19
3	1·13; 1·13	1·13; 1·14
4	1·10; 1·08	1·09; 1·13
5	1·10; 1·08	1·12; 1·10
6	nil	K_2SO_4 only present
6 + 1·2% of added KNO_3	1·22	—

TABLE II

Propellant	KNO_3 found polarographically %	KNO_3 calculated from analysis of of sulphated ash %
7	1·40	1·33
8	0·18	0·20
9	0·76; 0·78	0·84
10	2·40	2·37
11	nil	No KNO_3 present
11 + 0·5% of added KNO_3	0·50; 0·53	—
12	1·14	1·16
13	0·92	0·91
14	2·33	2·36
15	1·48	1·44

NOTES—(1) Pairs of figures are for duplicate estimations.
(2) The other essential constituents of the propellants were nitrocelluloses, nitroglycerine, diphenylamine, diethyldiphenylurea, phthalates, mineral jelly and graphite.

SUMMARY

The method proposed by Kolthoff, Harris and Matsuyama for the polarographic estimation of nitrate has been adapted to the estimation of nitrate in propellants. A method has been developed for destroying nitroguanidine, present in some propellants, which would otherwise interfere.

described offer simple, comparatively rapid and accurate means for the The in ganic nitrates in a wide range of propellants. With suitable choice of estimation could be used in routine specification analysis, for individual determinations procedurary, on extremely small samples. and, if

uthors wish to thank the Chief Scientist, Ministry of Supply, for permission to pub his communication.

REFERENCE

Kolthoff, I. M., Harris, W. E., and Matsuyama, G., *J. Amer. Chem. Soc.*, 1944, **66**, 1782.

LOSIVES RESEARCH AND DEVELOPMENT ESTABLISHMENT
MINISTRY OF SUPPLY
WOOLWICH, LONDON, S.E.18

April, 1948

The Determination of Small Amounts of Hydroquinone in Styrene

By S. M. A. WHETTEM

(Read at the Meeting of the Society, on Wednesday, November 3rd, 1948)

HYDROQUINONE is used to stabilise certain organic liquid monomers, such as styrene, which would otherwise polymerise to solids at ordinary room temperatures. It is often necessary to know precisely how much hydroquinone there is present in the stabilised material, or how much remains in the purified material.

The reduction of phosphomolybdate solutions by hydroquinone under certain specified conditions is the basis of a method by Straub[1] for the determination of phosphates in water and it seemed likely that this reaction could be applied to the determination of small amounts of hydroquinone. When this was found to be unsuccessful, attention was directed to the possible reduction of phosphotungstate solutions by the hydroquinone. The uric acid reagent of Folin and Denis,[2] as originally prepared, was found to be suitable. According to Wu[3] this reagent is phospho-18-tungstic acid.

EXPERIMENTAL

Solutions of hydroquinone were prepared containing from 0·01 to 0·10 mg. in 50 ml. of water. The first attempt to develop the molybdenum blue colour was made by adding to these solutions 2 ml. each of 20 per cent. v/v sulphuric acid, 10 per cent. w/v solution of A.R. ammonium molybdate and 10 per cent. w/v solution of A.R. diammonium phosphate, $(NH_4)_2HPO_4$. The colours produced were pale green and increased only slightly in intensity with the concentration of hydroquinone.

It was observed that if these solutions were made alkaline with sodium carbonate, the colours became more bluish and more intense. A second series of hydroquinone solutions was prepared containing the same amounts of hydroquinone in 40 ml. of water instead of 50 ml., to allow for subsequent addition of sodium carbonate solution. The same reagents were added as before and the green colour was developed for 15 minutes. At the end of that time the solutions were made alkaline with 10 ml. of 10 per cent. w/v sodium carbonate solution, and the colours measured in a Lovibond Tintometer, using 4-inch cells. The results, which are given in Table I, were promising in that the increase in colour intensity with the concentration of hydroquinone was more marked and the colours themselves more bluish and better to match.

It was considered, however, that the intensities were still not good enough to form the basis of a colorimetric method and attention was turned to the possibility of obtaining better results by using the phosphotungstate reaction. The sodium phosphotungstate reagent[2] was prepared as described below under REAGENTS.

To the same range of hydroquinone solutions, that is, 0·01 to 0·10 mg. of hydroquinone in 50 ml. of water, were added 2 ml. of the sodium phosphotungstate reagent and 4 ml. of 10 per cent. w/v sodium carbonate solution, making the total bulk of solution 56 ml., as in the previous experiments. The blue colours that developed were measured in the Tintometer, using 4-inch cells as before. The results are shown in Table I.

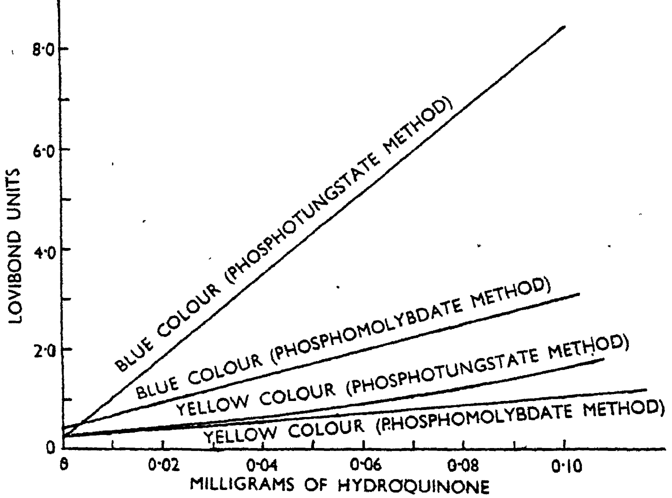
TABLE I

Hydroquinone mg.	Lovibond units							
	Phosphomolybdate				Phosphotungstate			
	(*a*)		(*b*)		(*a*)		(*b*)	
	Blue	Yellow	Blue	Yellow	Blue	Yellow	Blue	Yellow
Blank	0·4	0·2	0·4	0·2	0·2	0·2	0·2	0·1
0·01	0·6	0·4	0·6	0·4	1·0	0·3	1·0	0·3
0·03	1·1	0·5	1·1	0·5	2·6	0·5	2·6	0·5
0·05	1·6	0·6	1·7	0·6	4·3	0·7	4·3	0·7
0·07	2·1	0·7	2·1	0·7	6·1	1·0	6·0	1·0
0·10	3·0	1·0	3·1	0·9	8·4	1·6	8·0	1·4

(*a*) = readings taken 15 minutes after the addition of the reagents; (*b*) = readings after 3 hours.

These results show that, for the same concentrations of hydroquinone, the blue colour produced by the sodium phosphotungstate reagent is much more intense than that produced by the ammonium phosphomolybdate reagent. The yellow component is of about the same order in both methods and is comparatively small. The optimum time for colour development is approximately 15 minutes and the colours after 3 hours show only slight fading in the maximum part of the range.

By plotting graphs showing Lovibond units against milligrams of hydroquinone, it is seen that, in the range investigated, approximately linear relationships are obtained by both methods (see Fig.).



APPLICATION TO THE DETERMINATION OF HYDROQUINONE IN STYRENE—

The above experiments indicated that the Folin - Denis reagent, employed in the manner described, was suitable for the colorimetric determination of hydroquinone and might be applied to determining hydroquinone in styrene.

Solutions of freshly distilled styrene were prepared containing 0·01 to 0·10 mg. of hydroquinone per ml. of styrene. The hydroquinone in these solutions was extracted with water. The method used and the results obtained are given below.

METHOD

REAGENTS—

Sodium phosphotungstate solution—Boil together, under reflux, 10 g. of sodium tungstate (B.D.H. re-crystallised), 8 ml. of phosphoric acid (AnalaR) and 90 ml. of water for 1½ hours. Cool the solution and, if necessary, make up to 100 ml. with water.

Sodium carbonate solution—Prepare a 10 per cent. w/v aqueous solution of sodium carbonate A.R., filtering if necessary.

Standard solution of hydroquinone—Weigh out 1 g. of hydroquinone and dissolve in water in a 1-litre measuring flask. Make up to the mark with water and mix well. Dilute 100-fold for use. One ml. of this final solution ≡ 0·01 mg. of hydroquinone.

PROCEDURE—

Pipette 1 ml. of the sample of the styrene into a 50-ml. separating funnel. Extract the hydroquinone with 40 ml. of water, using 20 ml. for the first, 10 ml. for the second and 10 ml. for the third extraction. Collect the aqueous extracts in a 50-ml. Nessler cylinder and dilute with water to 50 ml. In similar Nessler cylinders prepare standards containing 1·0, 3·0, 5·0, 7·0 and 10 ml. of the standard hydroquinone solution and dilute each to 50 ml. To the sample and standards, add 2 ml. of the sodium phosphotungstate solution and 4 ml. of the sodium carbonate solution, *in this order,* mixing well after the addition of each reagent. Allow the solutions to stand for 15 minutes and compare the colour of the sample with that of the standards.

TABLE II

RESULTS ON STYRENE CONTAINING KNOWN AMOUNTS OF HYDROQUINONE

Hydroquinone added to 1 ml. of styrene mg.	Hydroquinone found mg.
0·010	0·010
0·030	0·030
0·050	0·047
0·070	0·070
0·100	0·096

Table II indicates that the method described for the extraction and determination of hydroquinone in styrene in these concentrations gives results within the experimental error and that the method is sensitive to 0·01 of a milligram of hydroquinone in 1 ml. of styrene or 10 mg. per litre. It can be made to detect 1 mg. per litre by extracting 10 ml. of styrene instead of 1 ml. with water.

RAPID METHOD FOR PROCESS CONTROL—

To avoid the necessity of making up standards for each determination, a colour disc is obtainable from Tintometer Ltd. containing 9 glasses, representing the range 0·01 to 0·09 mg. of hydroquinone, for use with the B.D.H. Lovibond Nessleriser. A "brightness screen" is also supplied with the disc, which is so placed on the top of the Nessleriser that the sample solution is viewed through the screen.

Treat the sample exactly as before, using one of the Nessleriser cylinders for the development of the blue colour. Prepare a reagent blank in the second cylinder by filling with water to the 50-ml. mark and adding 2 ml. of the sodium phosphotungstate solution and 4 ml. of the sodium carbonate solution. Place the sample solution in the right-hand side and the blank in the left-hand side of the Nessleriser. After 15 minutes have elapsed from the time of adding the reagents, match the colour of the sample with the colour glasses and read off the number of milligrams of hydroquinone shown on the disc.

INTERFERING SUBSTANCES—

Aqueous solutions of the following phenolic substances were prepared such that 1 ml. of each was equal to 0·01 mg. Five ml. of each solution were taken and made up to 50 ml. with water and the colour was developed in the same way as for hydroquinone. These colours were matched against the usual series of hydroquinone standards, with the following results.

Milligrams of substance present		Colour expressed as milligrams of hydroquinone
Phenol	0·05	None
ortho-Cresol	0·05	None
meta-Cresol	0·05	None
para-Cresol	0·05	None
Resorcinol	0·05	Trace (less than 0·01)
Pyrocatechol	0·05	0·09
Tannic acid	0·05	0·02

It appears, therefore, that of these substances, only pyrocatechol and tannic acid interfere seriously with the method.

HYDROQUINONE IN METHACRYLIC ESTERS—

Although the above investigation was carried out for the determination of hydroquinone in styrene, the method has been applied with success to methyl methacrylate monomer and other methacrylates.

SUMMARY

A method has been described for the determination of hydroquinone in styrene, which will determine concentrations down to 10 parts of hydroquinone per million of styrene w/v and can be modified to detect 1 part per million. Interference to be expected by related substances is detailed. The method has been applied with success to methacrylic esters. A rapid method using a Lovibond Nessleriser disc is also described.

The author wishes to thank Mr. Haslam for his valuable assistance in preparing this script, and Mr. Chamberlain of Messrs. Tintometer Ltd., for co-operating and supplying a suitable colour disc for the rapid method.

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IMPERIAL CHEMICAL INDUSTRIES LTD.
PLASTICS DIVISION
RESEARCH DEPARTMENT
WELWYN GARDEN CITY

DISCUSSION

The PRESIDENT asked how much hydroquinone is used to stabilise styrene.

Mr. W. C. WAKE said that hydroquinone and allied substances, which presumably could be estimated, are occasionally used, for research purposes, as anti-oxidants in rubber. One would like an assurance that oxidation products do not interfere.

Dr. W. ROMAN pointed out that other reducing agents would give the same reaction, and interfere; it was unlikely, however, that they would be present in styrene.

Dr. A. J. AMOS enquired as to the effect of changing the order in which the reagents are added to the test solution.

Mr. WHETTEM, in reply to the President, said that the amount of hydroquinone used to stabilise styrene was of the order of 0·01 per cent. In answer to Mr. Wake's question he said that quinone did not interfere with the reaction. Replying to Dr. Amos, he stated that if the sodium carbonate reagent was added before the phosphotungstate reagent no blue colour was produced.

Notes

THE USE OF REDUCED COPPER IN THE MICRO-DETERMINATION OF CARBON AND HYDROGEN

THE purpose of this note is to describe some preliminary attempts to avoid the use of lead dioxide in the combustion of nitrogenous organic compounds.

Niederl and Whitman[1] have described a method in which the substance, after being mixed with copper oxide, is burned in a stream of nitrogen. The following experiments were suggested by the modification of the micro-Dumas method devised by Dr. J. Unterzaucher[2] and successfully used in the laboratories of I.G. Farben, A.-G., Leverkusen. In this method the substance, contained in a platinum boat, is burned in a stream of carbon dioxide that has been bubbled through hydrogen peroxide to which a few scraps of platinum wire have been added to catalyse the production of oxygen. The oxygen transported by the carbon dioxide is sufficient to burn the sample, the excess being removed by a layer of reduced copper the greater part of which is not heated. In adapting the method to the determination of carbon and hydrogen, nitrogen was used as the transport gas; this was bubbled through a wash-bottle containing about 50 ml. of hydrogen peroxide and a few pieces of platinum wire. After passing through a flow-meter and a U-tube containing Ascarite and Anhydrone the gas was led into the side-arm of a silica micro-combustion tube.

The combustion tube contained an asbestos choking plug followed by 180 mm. of reduced copper and finally 150 mm. of copper oxide. The portion of the tube containing the reduced copper was covered by a metal sleeve and heated with a bunsen burner fitted with a flame spreader; the copper oxide was heated to redness by means of a small electric furnace.

The absorption tubes were of the normal Pregl type containing Anhydrone to absorb water and Ascarite to absorb carbon dioxide, and were weighed while filled with nitrogen.

Three to 5 mg. of material were weighed into a platinum boat and burned by the normal Pregl technique, taking about 20 minutes, during which time 120 ml. of gas were passed through the tube.

The introduction of silver into the combustion tube should make possible the analysis of substances containing halogen or sulphur, but owing to pressure of other work it has not yet been tried.

It is hoped to return to this investigation in the near future.

RESULTS

	Found		Calculated	
	% C	% H	% C	% H
Strychnine	75·66	6·69	75·45	6·58
	75·61	6·66		
Nitrobenzoic acid	50·61	2·98	50·30	2·98
	50·46	2·87		
	50·56	3·14		
Aminoacetic acid	32·17	6·66	32·00	6·66
	32·19	6·48		
Azobenzene	79·11	5·70	79·11	5·48
	79·29	5·64		

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1. Niederl, J. B., and Whitman, B., *Mikrochemie*, 1932, **11**, 274.
2. Unterzaucher, J., Private communication.

WELLCOME CHEMICAL RESEARCH LABORATORIES
BECKENHAM, KENT

A. BENNETT
August, 1948

ESTIMATION OF CYANIDE IN PLANT MATERIAL BY MEANS OF CONWAY UNITS

THE material on which this estimation was performed was a "feeding cake" imported from Turkey which had caused the death of several cows. The sample was found to be capable of producing considerable quantities of hydrocyanic acid and was most probably a bitter almond cake. Routine examination gave the following percentage figures: moisture at 100° C., 9·0; crude fat, 7·8; ash, 5·4; crude fibre, 5·8; protein (N × 6·25), 43·1; carbohydrates (as difference from 100), 28·9.

An attempt to determine the hydrocyanic acid by digestion at 60° C. for from 3 to 6 days, followed by aspiration of air in accordance with J. H. Roe's method,[1] proved unsatisfactory. Other methods found in the literature consulted[2] did not appear to be any more promising, and it occurred to us to try the use of Conway units,[3] which had already been found satisfactory for cyanides in general by Dr. Hirsch in these laboratories. The method finally adopted gave very satisfactory results and seems ideal for plant material, as the hydrocyanic acid liberated in the outer chamber of the unit is constantly absorbed in the inner chamber, thus enabling the decomposition of the glucoside to proceed to completion. The whole method is extremely simple, requiring no incubation and no aeration. Addition of tartaric acid was found unnecessary, though this might depend on the material analysed. At the end of an estimation all the units were kept for a further 24 hours to confirm that liberation of hydrocyanic acid was complete. The results obtained on the sample were 0·409 to 0·432 g. per cent. of hydrocyanic acid.

Recommended method—0·5 to 1·0 g. of the well-mixed and finely ground material is covered with 6 ml. of hot water at 60° C. If the material is too bulky for the Conway unit a squat weighing bottle (30 mm. by 60 mm., *Gallenkamp*, 11th Ed., No. 12,234) may be used, with a small Petri dish inserted in place of the middle chamber; it is advisable to keep the proportion between the sizes of the inner and outer chambers similar to that in Conway units so that the absorbing surface should be adequate. With the original Conway unit the maximum quantity of sample to be taken is 1 g., as the chamber is rather small. Into the central chamber 1 ml. to 2 ml. of 10 per cent. potassium hydroxide solution are introduced, and then the cover is smeared with yellow vaseline and the unit closed. It is allowed to stand at room temperature for 3 days, after which we found it advisable to add 2 ml. more of hot water at about 80° C. and allow it to stand for one more day. After addition of a few crystals of potassium iodide the liquid in the central chamber is titrated with 0·1 *N* or 0·02 *N* silver nitrate, according to the quantity of hydrocyanic acid expected. The end-point is sharp; 1 ml. of 0·1 *N* silver nitrate ≡ 0·0054 g. of HCN.

The accompanying table gives the results of a series of determinations.

Time allowed for decomposition, hours	Wt. of sample taken, g.	0·1 *N* $AgNO_3$ used, ml.	HCN found, %	Remarks
48	2	0·86	0·232	in squat weighing bottles
48	2	1·0	0·270	
72	2	1·32	0·356	
72	2	1·34	0·361	
72	2	1·25	0·337	
96	2	1·43	0·386	
96	2	1·43	0·386	
96	2	1·41	0·380	
96	2	1·43	0·386	
96	1	0·76	0·410	
96	0·5	0·38	0·410	
96	0·5	0·38	0·410	
		0·02 *N* $AgNO_3$ used, ml.		
96	1	3·78	0·409	in Conway units
96	1	3·78	0·409	
96	0·5	2·0	0·432	
96	0·5	1·96	0·423	
96	0·5	1·96	0·423	
96	0·5	1·94	0·419	
96	0·5	1·96	0·423	
96	0·5	1·94	0·419	
96	0·5	1·98	0·427	
96	0·5	2·0	0·432	
96	0·5	1·96	0·423	
96	0·5	1·96	0·423	
96	0·5	1·96	0·423	

As we decreased the quantity of the cake our results became higher, perhaps because of increased exposure of surface. At the same time, however, the experimental error also increased, a difference of only 0·04 ml. of 0·02 *N* $AgNO_3$ changed the results by 0·010 per cent. The mean figure obtained was 0·414 per cent. of hydrocyanic acid.

Summary—A method for estimating the cyanide content in plant material by means of the Conway unit is described. Almost constant results were obtained when 0·5 to 1 g. of samples was allowed to decompose for 96 hours in the closed unit.

Our thanks are due to Mr. H. R. Binns, M.R.C.V.S., for the loan of literature.

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3. Conway, E. J., *Micro-Diffusion Analysis and Volumetric Error*, Crosby, Lockwood & Son Ltd., London, 2nd Ed., 1947 (*cf. Analyst*, 1948, **73**, 419).
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DEPARTMENT OF HEALTH
GOVERNMENT OF PALESTINE
JERUSALEM

G. W. BAKER
S. TAUBES-STEINFELD

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1948, **73**, 679).

Public Analyst		*Appointments*
JAFFÉ, Frederick William Moore	..	County Borough of Dewsbury.
MCLACHLAN, Thomas (Deputy)	..	Royal Borough of New Windsor.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1948, **73**, 680).

Official Agricultural Analyst	*Appointments*
BRANSON, Victor Cecil (Deputy)	Administrative County of West Sussex. County Borough of Hastings.
JAFFÉ, Frederick William Moore	County Borough of Dewsbury.
SMITH, Arthur (Deputy)	County Borough of Kingston-upon-Hull.

Ministry of Food

STATUTORY INSTRUMENTS*

1949—No. 614. The Mineral Oil in Food Order, 1949. Price 1d.

This Order prohibits, from April 9th, 1949, *the use of mineral oil in the composition or preparation of any food sold or intended for sale for human consumption but does not apply where any article of food contains not more than* 0·2 *per cent. by weight of mineral oil and its inclusion is due to its use as a lubricant or greasing agent on some surface with which the article of food has necessarily to come into contact in the course of its preparation.*

"Mineral oil" means any hydrocarbon product, whether liquid, semi-liquid or solid, derived from any substance of mineral origin and includes liquid paraffins, white oils, petroleum jellies and hard paraffins.

Proceedings in England and Northern Ireland for an infringement of this Order may be brought by the Food and Drugs Authority without the consent of the Minister.

—No. 762. The Coffee Essence (Amendment) Order, 1949. Price 1d.

This Order, as from April 24th, 1949, *amends the Coffee Essence (Control) Order,* 1942, *as amended (S.R. & O.,* 1942, *No.* 560; *S.I.,* 1948, *No.* 1098), *by confining its application to dry coffee essences.*

—No. 781. The Chocolate, Sugar Confectionery and Cocoa Products Order, 1949. Price 3d.

This Order, as from April 24th, 1949, *revokes, consolidates and re-inacts the Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order,* 1944 (*S.R. & O.,* 1944, *No.* 451), *as amended (S.R. & O.,* 1945, *No.* 598; 1946, *Nos.* 1365, 1639 *and* 2233; 1947, *No.* 1927; *S.I.,* 1948, *Nos.* 233, 1962, 2470 *and* 2612; 1949, *No.* 190).

The principal changes, apart from prices, are—

Medicated chocolate and sugar confectionery is brought within the scope of the Order except for products that

- (a) *comply with recognised medical formulae; or*
- (b) *consist of compressed tablets each containing one or more of the following ingredients in not less than the amounts stated;*
  - (i) *phenolphthalein,* 0·5 *grain;*
  - (ii) *santonin,* 0·1 *grain;*
  - (iii) *calomel,* 0·05 *grain; or*
- (c) *are specifically exempted by the Minister.*

—No. 894. The Oils and Fats Order, 1949. Price 3d.

This consolidating Order revokes and replaces the Animal Fats (Control and Prices) Order, 1946, *as amended (S.R. & O.,* 1946, *No.* 2042; 1947, *Nos.* 557 *and* 2840), *the Edible Oils and Fats (Control of Sales) Order,* 1944, *as amended (S.R. & O.,* 1944, *No.* 672; 1947, *No.* 701), *the Oils and Oilseeds (Control) Order,* 1946 (*S.R. & O.,* 1946, *No.* 2043), *the Lard (Control and Maximum Prices) Order,* 1943, *as amended (S.R. & O.,* 1943, *No.* 1767; 1947, *No.* 2841) *and the Margarine and Cooking Fats (Requisition) Order,* 1940, *as amended (S.R. & O.,* 1940, *Nos.* 1107 *and* 1415).

The principal changes made are—

- (a) *Olive oil and sperm oil have been freed from all restrictions;*
- (b) *Sunflower seed and sunflower seed oil have been added to the list of oilseeds and oils that are subject to control;*
- (c) *A new definition of technical tallow has been included, viz.*

"Technical tallow" means any fat, grease or oil, other than premier jus, oleo oil, beef or mutton stearine, neatsfoot oil, or dripping, separated or substantially

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* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

separated by any process from the tissue or bones or from both tissue and bones of any quadruped animal other than the pig, and includes any mixture (other than any mixture containing more than 25 per cent. of mineral oil) of such fat, grease or oil with any other fat, grease or oil; but for the purpose of Articles 4 and 5 of this Order shall not include horse fat as defined herein, and for the purpose of Article 5 of this Order shall not include any oil having a melting-point which when determined by the method specified in the Fourth Schedule to this Order does not exceed 25 degrees centigrade.

Article 4, referred to in this definition, forbids the sale, etc., or use of any technical tallow or rendered pig fat other than lard, or any emulsion of technical tallow or rendered pig fat with any other substance, for use as human food or in the manufacture, preparation or cooking of human food.

Article 5 relates to maximum prices.

(d) *Among other definitions given in the Order are the following—*

"Cooking fats" means any fat or mixture of oils and fats fit for human food or for use in the manufacture, preparation or treatment of human food, having a melting-point which when determined by the method specified in the Fourth Schedule to this Order is not less than 30 degrees centigrade, but does not include dripping, premier jus, lard, margarine, butter, vegetarian butter, cocoa butter, horse fat, technical tallow, or any unrendered animal fat.

"Horse far" means unbleached, unadulterated oil or fat—(*a*) produced from, or by the rendering or processing of, the fat or bones of horses slaughtered for human consumption on premises licensed for such slaughter; (*b*) of sweet taste; (*c*) of sweet smell; (*d*) untreated by any chemical process; and (*e*) containing not less than 99 per cent. saponifiable matter and not more than 1·5 per cent. free fatty acids.

"Dripping" means unbleached, unadulterated fat—(*a*) produced from, or by the rendering or processing of, the fat or bones of sheep or oxen; (*b*) of a sweet taste; (*c*) of a sweet smell; (*d*) untreated by any chemical process; and (*e*) containing not less than 99 per cent. saponifiable matter and not more than 1·5 per cent. free fatty acids; but does not include imported premier jus.

"Lard" means rendered fat derived from a pig and having a melting point which when determined by the method specified in the Fourth Schedule to this Order is not less than 25 degrees centigrade, but (except for the purpose of Articles 2 and 9 of this Order) does not include unrefined or unbleached hog grease.

"Margarine" includes any vegetarian butter other than peanut butter or paste or sunflower butter or paste; it does not include cocoa butter.

"Vegetarian butter" means any soft fatty paste fit for human consumption consisting of vegetable oils or fats or a mixture of both, and containing also other material derived from oilseeds, nuts or kernels.

(e) *A list of oils, fats and mixtures that may be obtained only against a permit has been substituted for the general definition of the term "edible oil."*

(f) *The Margarine (Prepacking) Order, 1940 (S.R. & O., 1940, No. 898) is revoked and not re-enacted in this Order.*

Schedule IV states that for the purpose of this Order melting-points shall be determined with the apparatus and by the method of determining "drop-points" specified in British Standard No. 894: 1940—"British Standard Specification for the Determination of the Flow and Drop Points of Fats and Allied Substances (Apparatus and Method of Use)" as amended by Amendment No. 1, dated February, 1947; provided that for the purpose of Section B of the said British Standard, as amended, the cup referred to therein shall be filled, at a room temperature, with the fat to be treated without the application of heat to the sample.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Routine Method for the Determination of Diacetyl in Butter. P. C. Den Herder (*Netherlands Milk and Dairy J.*, 1947, **1**, 110–113)—The method of Prill and Hammer (*Iowa State Coll. J. Sci.*, 1938, **12**, 385) for determining diacetyl in butter, based upon steam distillation, is satisfactory but is too cumbersome for routine work. In molten butter diacetyl, if present, is dissolved partly in the fluid fat and partly in the serum. By passing a stream of carbon dioxide through the molten butter, first at 65° C. and then at 100° C. for a sufficiently long time, the whole of the diacetyl, together with the water present in the butter, is volatilised and can be collected in a receiver cooled in melting ice.

Procedure—The apparatus, with ground glass joints, consists of a distillation flask, into which a stream of carbon dioxide can be led, connected to two tubes in series acting as receivers. The carbon dioxide inlet tube reaches to the bottom of the flask and the delivery tube from the flask reaches to the bottom of the receiving tube. The second tube is always included, although it is unnecessary unless the diacetyl content of the butter is very high. The butter (25 g.) is placed in the flask, which has previously been filled with carbon dioxide. The first tube is charged with 1 ml. of the hydroxylamine solution described by Prill and Hammer (*loc. cit.*) and the second tube with 1·5 ml. of water. Both tubes are placed in melting ice. After connecting the carbon dioxide supply, the flask is placed in a water-bath maintained at 60° to 65° C., and a slow stream of carbon dioxide is passed through for 45 min. During this time most of the diacetyl with some of the water distils over. The water in the bath is now gently boiled and the carbon dioxide stream is accelerated. After about 30 min. the last traces of the diacetyl and practically all the water will have distilled over into the hydroxylamine in the first tube. The carbon dioxide stream is now stopped and the inlet tube of the first tube is rinsed with the water in the second receiver. The contents of the first receiver are transferred to a test tube, the second tube is rinsed with a little more water, which is then used to rinse the first tube and is added to the liquid in the test tube. The contents of the test tube are treated as described by Prill and Hammer (*loc. cit.*).

The accuracy of the method was established by adding known amounts of diacetyl to samples either prepared from sweet cream or freed from original diacetyl by steam distillation and determining the diacetyl content. Also, the results by this method were compared with those found by the steam distillation method (Prill and Hammer, *loc. cit.*). A. O. JONES

Determination of Alcohol-Insoluble Solids and Sugar Contents of Vegetables. J. C. Moyer and K. C. Holgate (*Anal. Chem.*, 1948, **20**, 472–474)—During a study of the losses of soluble constituents in the processing of vegetables, the need arose for a rapid method for determining the reducing and total sugars, and the desirability of combining the sugar determinations with that of the alcohol-insoluble solids became apparent.

The extraction of sugars from plant tissues with alcohol in sufficient concentration to precipitate polysaccharides and protein material and to inhibit enzymic action is greatly aided by maceration of the tissues in a Waring Blendor. The procedure gives a final alcoholic concentration of at least 75 per cent. according to the water content of the tissues.

Procedure—Freeze and store the material at −23° C. and, to obtain a representative sample, grind 1 lb. or more at this temperature twice in a food chopper. Weigh 20 g. of the finely ground sample and, at room temperature, wash it with 150 ml. of 85 per cent. alcohol into a Blendor cup of 500-ml. capacity having a rubber gasket under the screw top. After macerating for 5 min., wash the blended mixture into a 600-ml. beaker with 85 per cent. alcohol from a wash bottle, allow the solids to settle, and decant the liquid on to a weighed 5·5-cm., No. 40 Whatman filter paper in a Buchner funnel inserted through a two-holed rubber stopper in the mouth of a Kohlrausch sugar flask, to which suction can be applied by means of a tube passing through the other hole in the stopper. Transfer the mixture to the funnel and wash the solid matter on the filter three or four times with 85 per cent. alcohol, draining the residue after each washing, but avoiding thorough drying. When nearly 500 ml. of filtrate have been collected, allow the precipitate to dry, and place the paper in a weighing dish for complete drying overnight at 95° C. Finally, weigh the alcohol-insoluble solids. Adjust the volume of the filtrate to 500 ml. with 85 per cent. alcohol and mix thoroughly.

For clarification of the sugar solution the following modification of the procedure of Somogyi (*J. Biol. Chem.*, 1945, **160**, 61; *Analyst*, 1946, **71**, 85) was found suitable. Mix 56 g. of barium hydroxide with 2 litres of hot boiled water and filter the mixture into a storage bottle through a small Buchner funnel. The storage bottle should carry a soda lime tube and a siphon tube to a burette having a three-way tap. Dissolve 100 g. of zinc sulphate heptahydrate in 2 litres of water and adjust the concentration of the solution so that 10 ml. mixed with 50 ml. of water require 9·5 ml. of the barium hydroxide solution to give a faint pink end-point to phenolphthalein that is stable for 1 min. (The zinc sulphate and barium hydroxide solutions recommended by Somogyi leave zinc in the filtrate, which subsequently interferes with the colorimetric readings.) Evaporate 10 ml. of the alcoholic extract nearly to dryness on the steam-bath, rinse down the walls of the beaker with 5 ml. of water, add 2 ml. of barium hydroxide solution followed by 2 ml. of zinc sulphate solution with constant agitation during the addition of each reagent. Wash the liquid into a funnel of diameter 50 mm. and collect the filtrate in a graduated tube. Wash the precipitate with a fine stream of water until the filtrate measures 35 ml. For determination of reducing sugars use a 2-ml. aliquot of this filtrate, and for the determination of total sugars invert a 5-ml. aliquot.

An enzymic method of inversion was found better than an acid method. To prepare the buffer solution dissolve 13·6 g. of sodium acetate trihydrate in water, add 8 ml. of glacial acetic acid, and dilute to 500 ml. Dissolve 200 mg. of invertase scales (Wallenstein Laboratories, Blue Label) in 100 ml. of water and store the solution in an ice-box under a layer of toluene. To a 5-ml. aliquot of the clarified extract in a graduated tube add 2 drops of the acetate buffer and 5 drops of the invertase solution and invert overnight at 35° C. Dilute to 35 ml. with water and use a 2-ml. aliquot for colour development. The overnight incubation period has been used for convenience; a much shorter period at a higher temperature would doubtless accomplish the same degree of inversion.

The colorimetric procedure found suitable was that of Nelson (*Ibid.*, 1944, **153**, 375; *Analyst*, 1944, **69**, 313) in combination with Somogyi's new copper reagent (*loc. cit.*), but it was found that variations in the excess of arsenomolybdate reagent had less influence on the colour readings at 600 mμ. than at 500 mμ. as recommended by Nelson.

To prepare Somogyi's copper reagent add 56 g. of anhydrous disodium phosphate slowly with stirring to 1400 ml. of water, and then with continued stirring 80 g. of Rochelle salt and finally 200 ml. of *N* sodium hydroxide. Dissolve 16 g. of copper sulphate in 160 ml. of water and add this solution to the first. Finally, add slowly, 360 g. of anhydrous sodium sulphate with stirring, dilute to 2 litres, and set the solution aside for 2 days before filtering. To prepare Nelson's arsenomolybdate reagent, dissolve 100 g. of ammonium molybdate with stirring in 1800 ml. of water, add 84 ml. of concentrated sulphuric acid with continued agitation and 12 g. of sodium arsenate heptahydrate. Finally, dilute the solution to 2 litres, set it aside for 48 hr. at 37° C., filter, and store it in a brown bottle.

Procedure—Place a 2-ml. aliquot of the clarified extract (for reducing sugars) or of the inverted extract (for total sugars) in a Folin-Wu blood sugar tube by means of an Ostwald pipette. Then add 2 ml. of Somogyi's copper reagent from a 25-ml. burette and place the tube in boiling water for 20 min. Cool the tube in water at room temperature, add 2 ml. of Nelson's arsenomolybdate reagent and mix the solutions by moderate agitation with a glass rod having a small knob at the end. Wash the rod and dilute the liquid to 25 ml. before shaking to ensure thorough mixing. Allow the solution to stand for 15 min. and read the colour intensity at 600 mμ. in a photo-electric colorimeter or spectrophotometer that has been adjusted to give 100 per cent. transmittance with water. With each series of unknown samples treat tubes containing 2 ml. of water for the blank value and 2 ml. of standard solutions containing 0·10 and 0·20 mg. of glucose in a similar manner to obtain a standard reference curve.

The precision of the analyses was determined with ten replicate samples of ground frozen lima beans, in which the reducing sugar content was found to be 0·068 per cent. and the standard deviation of a single determination ±0·003. After inversion of aliquots of the same extracts the total sugar content was 2·43 per cent. with ±0·07 as the standard deviation of a single determination. A. O. JONES

Specific Surface of Wheat Flours. I. Determination by Air Permeability Method. L. Lyon, N. Pennington, and A. Boley (*Cereal Chem.*, 1947, **24**, 394–407)—The method used is based on the determination of the rate of flow of air through a plug of the sample under a measured difference of pressure between the ends of the plug. The theory of the method has been worked out by Carman (*Trans. Inst. Chem. Eng.*, 1937, **15**, 150–166; *J. Soc. Chem. Ind.*, 1938, **57**, 225–234T; 1939, **58**, 1–7T) and by Sullivan and Hertel ("*Advances in Colloid Chemistry*," Vol. I, pp. 37–80).

The specific surface is said to be expressed by the equation

$$S = \frac{14}{d_s}\sqrt{\frac{A\Delta Pt}{Q\eta L}\cdot\frac{\epsilon^3}{(1-\epsilon)^2}} \quad \cdots \quad (1)$$

in which S = Specific surface of the powder in sq. cm. per gram.
d_s = Density of the particles of the powder.
A = Cross-sectional area of the plug in sq. cm.
L = Length of the plug in cm.
W = Weight of the plug in grams.
ϵ = Porosity of the plug = $1 - (W/d_sAL)$.
η = Viscosity of the air in poises.
Q = Volume of air flowing through the plug in t seconds.
ΔP = Pressure difference of air between ends of plug, in grams weight per sq. cm.

Apparatus and Procedure—The essential features of the apparatus are shown in Fig. 1. The kerosene manometer F measures the difference of air pressure at the two end of the plug, A, and the system B, C, D, E, measures the rate of air-flow through the plug. The sequence of operations is as follows. With the three-way stopcock B open to the air, a pressure difference is established across A. After steady flow has been attained, usually in 3 min., B is closed and air from system D is drawn through A. As the pressure in D is lowered, kerosene from C flows into D, replacing the air. The kerosene level in C is adjusted so that a lowering of pressure approximating to 1 mm. of kerosene in D causes the liquid in C to flow into chamber D. Thus the pressure measurement by manometer F is not affected appreciably by the volume measurement. The rate of flow of air through A is determined by measuring the time taken for the kerosene to fill the space in container D defined by two etched marks on a capillary side-arm. In the apparatus used that space was 4·977 ml. When the determination is complete the kerosene is drained from D by stopcock E and returned to reservoir C.

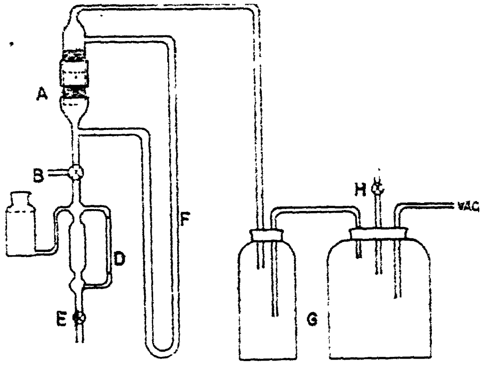


Fig. 1. Air permeability apparatus. A, sample tube. B, three-way stopcock. C, kerosene reservoir. D, volume measurement device. E, two-way stopcock. F, kerosene manometer. G, surge bottles. H, vacuum regulator.

The plugs of flour to be tested are formed in machined brass tubes of accurately known dimensions in which they remain during the test. Ten tubes, ranging in length from 20 to 40 mm. and in internal diameter from 4 to 12 mm., were in use. To ensure uniformity of packing throughout the length of a tube a tamping apparatus (Fig. 2) is used. The sample tube is screwed into its base, about 0·1 g. of the flour is introduced at a time and each portion pressed by means of a plunger, of diameter 0·002 in. less than the inner diameter of the tube, on which a gravity-hammer is allowed to fall a certain number of times from a predetermined height, according to the degree of packing required. The weight of the plug is found by weighing the tube before and after filling.

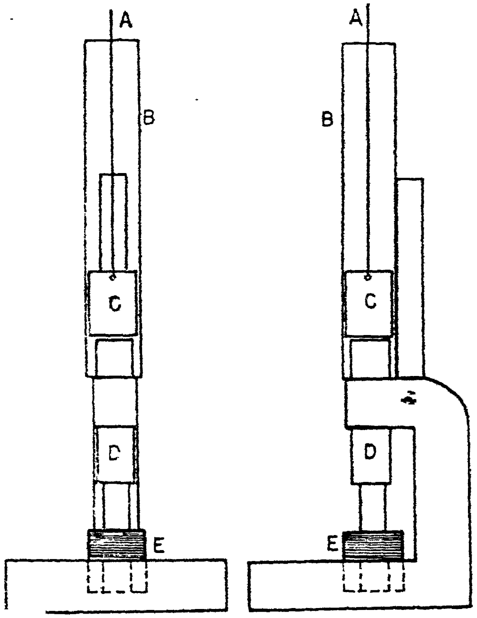


Fig. 2. Tamping apparatus. A, wire handle. B, glass guide tube. C, bucket containing lead shot. D, steel plunger. E, machined sample tube.

If necessary the density of a flour is determined in a 25-ml. pycnometer with xylene as liquid, at 25° C., but it is rarely outside the range 1·42 to 1·45, and for routine tests the latter figure is usually taken.

Influence of experimental factors—In equation (1), which may be written in the form

$$\frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S^2 d_s^2 QL}{14^2 A} \cdot \frac{\eta}{\Delta Pt} \quad .. \qquad .. \quad (1a)$$

it was found that, for a range of ΔP from 0·2 to 100 cm. of kerosene, the product ΔPt is constant to within 1 per cent. if all other factors are kept unchanged.

The influence of other factors was studied in experiments with a hard winter wheat. By adding to the right-hand side of equation (1*a*) a correction term, *b*, better agreement with experimental results was obtained for plugs of the same size, the standard error of the *S* values calculated from the modified equation (equation 2) being 2·6 per cent. compared with 10 per cent. for those calculated from equation (1) or (1*a*).

By varying the size of plug used it was shown that *S* was dependent on the length (*L*) and cross-sectional area (*A*) of the plug, a minimum value for *S* being obtained when A/L was approximately 0·45. The equation was accordingly modified into its final form,

$$\frac{A}{L} \quad \frac{\epsilon^3}{(1-\epsilon)^2} \qquad S^2 \cdot \frac{d_s^2 Q\eta}{14^2 \Delta Pt}$$

where *C* is a correction term, which depends on the size of plug (*i.e.*, of tube) used and the value of *S* to be determined. For a given sample (*i.e.*, *S* constant) and a given size of plug, it may be determined by carrying out several determinations and plotting the values of $A\epsilon^3/L(1-\epsilon)^2$ and $10^9 \times d_s^2 Q/14^2 \Delta Pt$ The graph is a straight line, whose intercept on the axis of $A\epsilon^3/L(1-\epsilon)^2$ is *C* and slope is S^2. The accompanying table shows the specific surface of a patent flour determined with ten sizes of plug (tube) and calculated by use of equation (3) and the appropriate values of *C*: for each tube (plug size) the first figure is the length and the second the diameter, in mm.

Tube	$\sqrt{A/L}$	*S*, cm.^{2}	*C*
20–4	0·253	2170	0·0016
40–6	0·264	2180	0·0018
20–5	0·297	2010	0·0032
30–6	0·305	2020	0·0038
20–6	0·373	1980	0·0047
20–8	0·504	1960	0·0115
40–12	0·530	1980	0·0138
30–12	0·612	1960	0·0175
20–10	0·616	1950	0·0200
20–12	0·745	1950	0·0340

Average of last 6 tubes: 1963

Provided that $\sqrt{A/L}$ is greater than about 0·375, *S* is independent of $\sqrt{A/L}$ to within 1 per cent. When $\sqrt{A/L}$ is greater than 0·4, results are obtained with a standard error of 0·5 per cent.

At least two determinations are necessary to find S by this method. To evaluate S by one determination the dependence of C on S must be known, for the plug size used (*cf.* Fig. 3); an approximate value of S is first calculated by means of equation (1) and the corresponding correction term estimated from a graph such as Fig. 3 and inserted in equation (3) to enable a corrected value of S to be obtained.

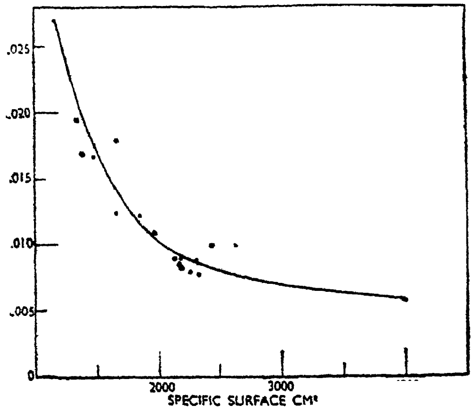


Fig. 3. Variation of correction term C with specific surface. Sample tube 20–8.

Comparison of the corresponding diameter, D, of equivalent smooth spheres ($D = 6/Sd_s$) with the particle size results obtained by sedimentation methods indicates that the equivalent diameters found by surface area measurements are smaller than those obtained from sedimentation or microscopical measurements. This is undoubtedly due to the irregular shape of flour particles.

Results—The following table shows the results of specific surface determinations on fractions of patent flour obtained by using A.S.T.M. wire sieves (in Ro-Tap shaker for 10 min.).

A.S.T.M. sieves	Weight, grams	Specific surface, S in cm.²	Diameter, D in microns	Total surface area, grams × S
Over 80	0·136	—	—	—
Over 100	10·958	1380	29·9	15·120
Over 140	8·239	1350	30·6	11·100
Over 180	5·339	1660	24·9	8·850
Over 300	23·400	2440	16·85	57·300
Under 300	0·459	—	—	—
Totals	48·531			92·370

Total weight exclusive of "over 80" and "under 300" = 47·936 g.

$S = \frac{92{\cdot}370}{47{\cdot}936} = 1930$ cm.², the average specific surface from the above sieve analysis.

$S = 1960$ cm.², the average specific surface as determined on original flour.

W. Martin

Methods for Determining Flour Particle-Size Distribution. F. W. Wichser and J. A. Shellenberger (*Cereal Chem.*, 1948, 25, 155–167)—The three methods dealt with in the present paper are those depending on the use of sieves, air elutriation, and sedimentation, respectively. All results were obtained with the same flour. Sieve methods using special wire sieves having accurately known apertures (*e.g.*, Tyler standard screen scale testing sieves) can give reproducible and accurate results for particles of size greater than 37 mμ. with the Ro-Tap shaker. The curve marked "sieve" in Fig. 1 shows a typical result for wheat flour by this sieve method (single sieve procedure), operated according to the procedure of Wichser *et al.* (*Ibid.*, 1947, **24**, 381–393).

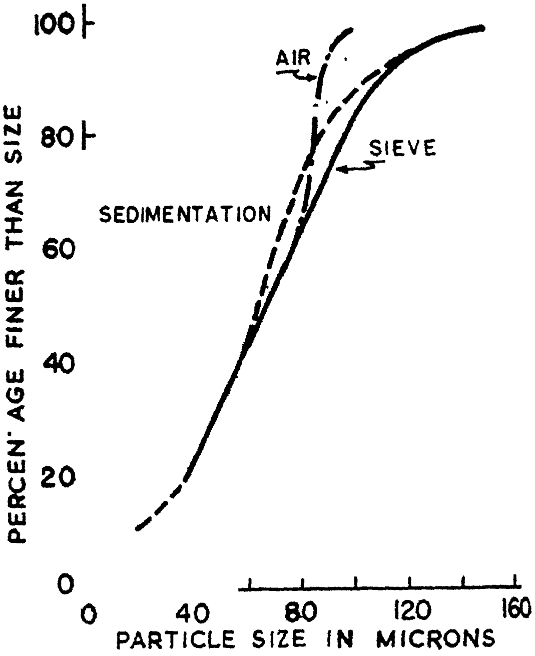


Fig. 1—Comparison of particle-size distribution curves for wheat flour by sieving, air separation, and sedimentation.

The air elutriation method employed was the Roller Particle Size Air Analyser described by Wichser *et al.* (*loc. cit.*). This method depends on the systematic removal of particles by increasing air velocity in a suitable expansion chamber. Stokes's law in the form given below can be applied to this method.

$$V = 10^{-8}gDd^2/18\eta = 0{\cdot}00299\, Dd^2 \text{ cm. per sec.},$$

where V = terminal velocity of fall in cm. per sec. in stationary fluid, g = gravitational constant (980·3 dynes), D = density of particles in g. per ml., η = viscosity of fluid in c.g. units = $1{\cdot}82 \times 10^{-4}$ for air, and d = diameter of particles in microns. The curve marked "air" in Fig. 1 shows the result obtained by this method.

The sedimentation method investigated made use of the Andreasen pipette shown in Fig. 2. Andreasen (*Kolloid-Z.*, 1929, **48**, 175) showed that Stokes's law could be applied to angular particles of the same weight as spherical particles. By calculating the particle size as the edge length of a cube of the same volume as a sphere of radius r, his particle size conformed to the result of sieve analysis. This expression is shown as

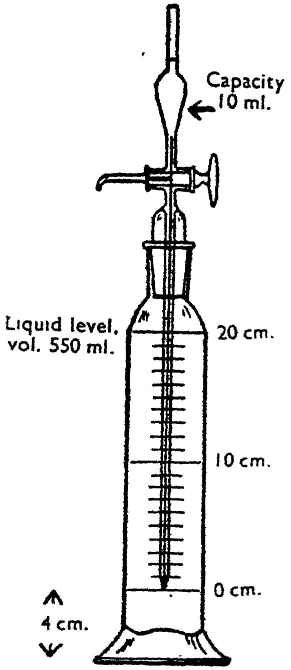


Fig. 2

$$\left[2739\sqrt{\frac{nh}{(D-d)tg}}\right]1{\cdot}612,$$

where r = edge-length of particle in microns, n = viscosity of suspending medium in poises, h = height in cm. between liquid surface and pipette tip when sample is drawn, t = time in minutes of settling, D = density of flour, and d = density of suspending medium.

Substituting in the above equation the constants of the test, it is reduced to the form $r = k\sqrt{h/t}$, where k is a constant for all the known values.

The medium used was a mixture of naphtha and carbon tetrachloride. 6-g. portions of flour were added to the suspending medium in the pipette and the whole was well shaken to get a uniform mixture. An initial sample was taken and the apparatus kept at 30° C. Subsequently, 10-ml. portions were removed at suitable time intervals and the solids determined by evaporating the liquid. The curve marked "sedimentation," obtained by this method, is shown in Fig. 1.

The results obtained show that the sieve method using single sieves as opposed to stack sieves is the most accurate and trustworthy method for the purpose. The air elutriation method is comparably rapid, but is expensive in outlay and is limited to the smaller size particles. The sedimentation method has too many disadvantages both in operation and in determining the necessary constants. W. MARTIN

Determination of Theobromine and Caffeine in Cacao Materials. R. G. Moores and H. A. Campbell (*Anal. Chem.*, 1948, **20**, 40–47)—THEOBROMINE—For the greatest accuracy, sample weights should be adjusted so that the materials in the clarification and adsorption steps contain about 40 mg. of theobromine. *Procedure*—To 2 to 3 g. of the sample add 1 g. of heavy magnesium oxide (U.S.P.) and about 4 g. of Celite 545, make into a smooth paste with hot water, mix thoroughly, and transfer the mixture to an extraction tube made by attaching a 100-ml. length of 8-mm. glass tubing to a 25 × 200-mm. test tube containing a glass wool plug and a Celite 545 filter bed (about 1 g.) fitted into a litre filter flask. Connect the extraction tube to a supply of boiling water and apply suction to the flask so as to draw boiling water through at such a rate that 500 ml. of extract will be collected in 30 to 40 min. The tube should not become dry during the percolation, and, to maintain the temperature of the sample at 80° to 90° C., the water should not be more than 2·5 cm. above the sample. Adjust the pH of the extract to about 6 by means of N sulphuric acid and Universal Indicator paper and concentrate it to about 150 ml. Rinse the concentrate into a 200-ml. flask with small amounts of water until the volume is about 170 ml. Add 7 ml. of zinc acetate reagent (219 g. of zinc acetate dihydrate dissolved in water with 30 ml. of glacial acetic acid and diluted to 1 litre) and then immediately, with swirling, add 7 ml. of potassium ferrocyanide solution (106 g. of potassium ferrocyanide trihydrate in 1 litre of water). Adjust the volume to 200 ml. and mix thoroughly. After not less than 3 and not more than 5 min. filter the liquid through a dry paper (Green's No. 4882) and collect all but the first 5 to 8 ml. of the filtrate. Place 150 ml. in a separating funnel equipped with a rubber stopper to fit the top of the adsorption tube, which is made by attaching 80 mm. of 6-mm. glass tubing to the bottom of an 18 × 100-mm. test tube.

Place a glass-wool plug firmly in the bottom of the adsorption tube, add about 0·5 g. of Celite 545 to make a bed about 10 mm. thick and above it place about 6 g. of an intimate mixture (1 + 1) of Celite 545 (or 535) and English Superfine XL fuller's earth. The fuller's earth should be tested for its adsorptive capacity by the following test. Stir 2 g. for 10 min. with a neutral aqueous solution of 200 mg. of theobromine in 200 ml. Centrifuge and determine the theobromine in an aliquot by the silver nitrate titration procedure (*infra*). The fuller's earth should adsorb at least 75 mg. of the theobromine.

Compress the packing by drawing air through the dry bed, and ensure that the column is free from channels. Immediately connect the adsorption tube to the separating funnel containing the clarified solution, which should contain preferably 40 mg. and not more than 60 mg. of theobromine. Draw the solution through the column in 20 to 30 min. until only 4 to 5 ml. remain above the clay bed. Wash the column with 50 ml. of water, keeping some liquid above the column at all times.

Replace the suction flask by a clean one, place 75 ml. of N sodium hydroxide in the separating funnel, and elute the theobromine by connecting the funnel to the adsorption tube and drawing the alkali through the column by suction. The time for elution should be 10 to 20 min. Add 2 drops of a mixed indicator prepared by dissolving 0·625 g. of methyl red in 450 ml. of 95 per cent. alcohol, filtering the solution through asbestos, mixing it with 0·412 g. of methylene blue in 50 ml. of water and diluting the mixture to 500 ml. The pH range of the indicator is 5·34 to 5·65, and the colour change from reddish-purple to green. Neutralise the eluate with N sulphuric acid to the faint red end-point and transfer it to a 250-ml. beaker, rinsing the flask with water. In the beaker place a glass electrode and a calomel electrode making contact with the solution by means of a salt bridge made by sealing a thread of asbestos into the end of an 18 × 100-mm. test tube filled with a saturated solution of sodium nitrate. Adjust the pH to 6·4 ± 0·05, using 0·025 N sodium hydroxide for the final adjustment. The total volume should now be about 125 ml. Add 25 ml. of 0·01 N silver nitrate, mix thoroughly and, with vigorous stirring, titrate to pH 6·40 ± 0·05, thereby determining the nitric acid liberated by formation of silver theobromine. For best results the titration should be made without interruption and within 5 min. Submit a sample of water to the processes of clarification, adsorption, elution with sodium hydroxide, neutralisation, and titration in presence of silver nitrate exactly as described. This blank determination should require not more than 0·2 ml. of 0·025 N sodium hydroxide. Submit a sample containing 40 mg. of theobromine to the same procedure to establish the quantitative nature of all the steps involved. The percentage of theobromine is given by $100\,nf/w$, where n is the difference between the titre of the sample and that of the blank, f is the theobromine factor and w is the weight of sample clarified. The theobromine factor is $0{\cdot}18\,Nv/a$, where N is the normality of the sodium hydroxide solution, v is the total volume clarified and a is the aliquot analysed. For 0·025 N sodium hydroxide and a 150-ml. aliquot from a total volume of 200 ml. clarified the factor is 0·006. A survey of the results of comparative analyses, recovery experiments, and about 10,000 determinations by the method indicates that for the highest accuracy an empirical correction of about 3 per cent. should be added to all results from samples containing soluble cacao materials. The correction for pure solutions of theobromine carried through the entire procedure should be 5 per cent.

With pure or crude theobromine samples the clarification and adsorption steps can be omitted. To 0·1 g. of the pulverised sample in a 500-ml. Erlenmeyer flask add 250 ml. of water, place a small funnel in the neck, and boil for 30 min. Filter while still hot through coarse paper into a 400-ml. beaker. (Filtration may be omitted with samples containing 90 to 100 per cent. of theobromine.) Wash the filter paper thoroughly with 50 ml. of hot water, cool the filtrate and washings to room temperature, adjust to pH 6·4 with 0·025 N sodium hydroxide, add silver nitrate, and titrate back as already described. Correct the result by means of a blank, using 250 ml. of water and 5 ml. of silver nitrate solution.

Chlorides and sulphates do not interfere with the titration unless they are present in amount sufficient to alter the concentration of the silver nitrate. If a large amount of chloride is present, the silver nitrate concentration should be increased by an equivalent amount. Carbonates interfere by formation of silver carbonate with consequent liberation of nitric acid which appears as an apparent increase in the theobromine content. The sodium hydroxide solutions should therefore be prepared from a 50 per cent. solution that has been allowed to stand for 10 days. Traces of carbonate occurring in the reagents will be compensated for in the blank determination.

CAFFEINE—*Procedure*—Mix 4 g. of the sample with 2 g. of heavy magnesium oxide and 8 g. of Celite 545 and extract it by the procedure described for theobromine. Neutralise the extract, concentrate it to 150 ml. and clarify it with zinc ferrocyanide. Add 10 ml. of 0·5 M sodium phosphate (190 g. of trisodium phosphate dodecahydrate in a litre of water) to 150 ml. of the filtrate, dilute to 200 ml., and filter through fluted paper.

Extract 150 ml. of the filtrate five successive times for 1 min. each with 30-ml. portions of chloroform and to the combined extracts in a separating funnel add 5 ml. of N sulphuric acid, mix thoroughly, allow to stand for 10 min., and draw off the chloroform layer through a cottonwool plug in the stem of the separating funnel into a 650-ml. Kjeldahl flask. Wash the residual acid layer with 30 ml. of chloroform and run the washing into the Kjeldahl flask. Remove all but 10 to 15 ml. of the solvent by slow distillation and determine the total nitrogen in the residue by the Kjeldahl-Gunning method. Make a blank determination with the reagents used, starting with 180 ml. of chloroform. The percentage of caffeine is given by $4{\cdot}85\,nN/w$, where n is the volume of sodium hydroxide solution of normality N (corrected for the blank titration) used and w is the weight of sample.

The recommendations of Wadsworth (*Analyst*, 1921, **46**, 32; 1922, **47**, 152) for the preparation of cacao materials for analysis are satisfactory. Samples of whole beans or nibs containing more than 15 per cent. of fat should be treated with a fat solvent (*e.g.*, hexane or pentane) to facilitate extraction and subsequent clarification and adsorption.

A. O. JONES

Use of Low-Temperature Crystallisation in the Determination of Component Acids of Liquid Fats. IV. Marine Animal Oils. Component Acids and Glycerides of a Grey Atlantic Seal. T. P. Hilditch and S. P. Pathak (*J. Soc. Chem. Ind.*, 1947, **66**, 421–424)—The component fatty acids of grey seal blubber oil were separated into four groups: A, insoluble in ether at −30° C.; B, soluble in ether at −30° C.; C, soluble in acetone at −40° C.; D, soluble in acetone at −60° C. The following results were obtained:—

Component acids (increment per cent. wt.)	A (11·4)	B (17·1)	C (28·3)	D (43·2)	Total	%, excluding unsaponifiable (wt.)	%, excluding unsaponifiable (mol.)
Myristic	1·3	1·3	0·7	0·4	3·7	3·7	4·6
Palmitic	8·3	1·4	0·8	—	10·5	10·5	11·5
Stearic	1·4	0·6	—	—	2·0	2·0	2·0
Unsaturated C_{14}	—	0·1	0·6	0·9	1·6	1·6	2·1
" C_{16}	0·1	1·6	5·8	7·9	15·4	15·5	17·2
" C_{18}	0·3	10·1	12·3	8·0	30·7	30·8	30·9
" C_{20}	—	1·9	7·1	7·4	16·4	16·5	15·2
" C_{22}	—	—	0·9	17·0	17·9	18·1	15·5
" C_{24}	—	—	—	1·3	1·3	1·3	1·0
Unsaponifiable matter ..	—	0·1	0·1	0·3	0·5	—	—
Iodine value (per cent.) ..	2·8	75·6	103·1	294·4			

Mixed glycerides fractionated from acetone were:—

	Description	Per cent. (wt.)	Iodine value	Saponification value
A	Insoluble at −10° C.	16·4	73·9	282·4
B	Soluble at −10° C. or −15° C., insoluble at −40° C.	28·9	119·7	287·7
C	Soluble at −40° C.; insoluble at −40° C. ..	30·4	189·6	295·1
D	Soluble at −60° C.	24·3	241·8	303·5

	A	B	C	D	Total
Glycerides per cent. (mol.) ..	17·0	29·5	30·1	23·4	100·0

The composition of these was:—

	Component acids per cent.									
	A		B		C		D		Total	
	(wt.)	(mol.)	(wt.)	(mol.)	(wt.)	(mol.)	(wt.)	(mol.)	(wt.)	(mol.)
Myristic	6·0	7·1	4·3	5·2	4·1	5·0	1·8	2·3	3·9	4·7
Palmitic	29·8	31·3	12·8	13·6	6·2	6·9	2·2	2·5	11·0	12·1
Stearic	6·3	6·0	2·2	3·1	0·9	0·9	0·6	0·6	2·1	2·0
Arachidic	0·5	0·4	0·1	0·1	—	—	—	—	0·1	0·1
Unsaturated C_{14} ..	1·2	1·4	3·0	3·6	2·8	3·5	3·4	4·3	2·7	3·3
" C_{16} ..	13·0	13·8	20·7	22·2	17·0	18·9	17·3	19·7	17·5	19·2
" C_{18} ..	17·9	26·6	38·2	37·0	32·5	32·6	27·3	28·1	32·2	31·8
" C_{20} ..	15·1	13·2	12·8	11·4	15·8	14·6	12·5	11·9	14·0	12·9
" C_{22} ..	0·2	0·2	5·9	4·8	19·5	16·7	34·0	29·8	15·9	13·4
" C_{24} ..	—	—	—	—	1·2	0·9	0·9	0·8	0·6	0·5

The glycerides are the extremely "mixed" type characteristic of marine animal oils. Liver tissue examined contained less than 3 per cent. of lipids, consisting of phosphatides (29 per cent.), unsaponifiable matter (21 per cent.), and glycerides (50 per cent.); the liver glycerides contained a less unsaturated mixture of fatty acids than the blubber glycerides, and also a higher content of saturated fatty acids.

E. B. DAW

Content of Morphine and other Alkaloids in Poppy Heads of Different Varieties. J. F. Reith, A. W. M. Indemans, and W. H. Becker (*Pharm. Weekblad.*, 1948, **83**, 449–459)—In 1946 an examination was made of the alkaloidal content of poppy capsules of different varieties grown in Holland. The results were reported in the *Pharm. Weekblad*, 1947, **82**, 582. The plan of the work comprised the following points. (1) Observation and analysis of 51 foreign varieties. (2) Comparison of the yields of seed, capsule, and alkaloids of four Dutch, four German, and eight French varieties. (3) Comparison of the results obtained with four Dutch varieties grown at four different places. (4) Continuation of the selection tests of the preceding year. The table below summarises the results of these tests and gives figures for the outstanding varieties.

		Per cent.	
Origin		Morphine	Other alkaloids
Series I:			
Denmark	Mean of 10 varieties	0·54	0·11
	"Aesbo 9"	0·62	0·09
Germany	Mean of 3 varieties	0·49	0·16
Lithuania	Mean of 3 varieties	0·42	0·31
	"Lithaus red"	0·28	0·45
Turkey	"Amasya"	0·73	0·21
Czechoslovakia	Mean of 3 varieties	0·43	0·11
	"Dubsky Stribosedy"	0·59	0·16
Hungary	"Hongaars 2"	0·42	0·12
Austria	"Prohasha"	0·35	0·07
Sweden	Mean of 28 varieties	0·43	0·21
	Pap. somn. alb. Kaunas × Pap. somn. var. opif. Kaunas	0·33	0·56
	Cross P36	0·62	0·28
Series II:			
Holland	Mean of 4 varieties	0·50	0·09
Germany	Mean of 4 varieties	0·49	0·14
France	Mean of 8 varieties	0·55	0·29

The yields of seed in the second series were low, but weather conditions were abnormal. The best figure was 930 kg. per hectare for the Dutch "Noordster" strain. Differences were observed in the alkaloidal contents of plants grown in different parts of the country, but some strains appeared to be affected more than others by a change in locality. The morphine contents in 1947 were higher than in the preceding year, possibly owing to the drought. The figures indicate that selection of seed from capsules with a high alkaloidal content has favourable results. Considerable differences were found in the molecular weights of the "other alkaloids" obtained in the analysis. This mean molecular weight decreased after purification by silicotungstic acid. G. Middleton

Biochemical

Determination of Aspartic Acid. B. Warshowsky and M. W. Rice (*Anal. Chem.*, 1948, **20**, 341–344)—In view of the difficulty of carrying out a quantitative isolation of aspartic acid from protein hydrolysates, a method for its determination in presence of other amino acids has been worked out. The aspartic acid is converted to a mixture of maleic and fumaric acids which are determined polarographically (*cf. Analyst*, 1948, **73**, 45).

Procedure—To a sample containing between 2 and 10 mg. of aspartic acid in 2 to 5 ml. of solution, in a 50-ml. conical flask, add about 2 ml. of dimethyl sulphate followed by several drops of phenolphthalein indicator solution. Make the solution just alkaline by adding 40 per cent. sodium hydroxide solution drop by drop, and place the flask in a water-bath below 25° C., adding more sodium hydroxide at frequent intervals to keep the solution alkaline. It is necessary to add further amounts of phenolphthalein during this time as the indicator is gradually destroyed. After 1½ hr., add 1 ml. of concentrated sulphuric acid and allow the mixture to stand for a further ½ hr. Neutralise the solution with the sodium hydroxide solution, and transfer it to a 100-ml. volumetric flask. Add sufficient 6·0 *N* hydrochloric acid to reduce the pH to 1·5 (about 2 ml.), dilute to 100 ml. and, after removing dissolved oxygen, examine a portion of the solution polarographically over the range −0·5 to −1·1 v. *versus* the mercury pool. The wave-height obtained gives the aspartic acid content of the solution by reference to a calibration curve obtained by treating solutions of various known concentrations of aspartic acid by the same technique.

Experiments in which known amounts of aspartic acid were added to protein hydrolysates gave an average recovery of 99·5 per cent. J. G. Waller

Estimation of Creatinine. J. A. Barclay and R. A. Kenney (*Biochem. J.*, 1947, **41**, 586–589)—A nephelometric method that is more selective than the Folin technique is described for the estimation of creatinine. The method is a modification of that suggested by Barrett (*Lancet*, 1936, **1**, 84) using Nessler's solution to which 25 ml. of 10 per cent. potassium iodide solution has been added per 100 ml.

Procedure—Dilute the sample to contain less than 3 mg. of creatinine per 100 ml. Add 3 ml. of this solution to 1 ml. of the reagent. Mix and allow to stand for 5 min. The precipitate remains dispersed for 30 min. Estimate nephelometrically in a photo-electric absorptiometer with a Chance O.B.2 glass light filter. Compare with standard solutions covering a suitable range not exceeding 3 mg. of creatinine per 100 ml., treated similarly.

Blood and plasma samples—De-proteinise by the zinc hydroxide method of Somogyi (*J. Biol. Chem.*, 1930, **36**, 655) or the tungstic acid method of Folin and Wu (*Ibid.*, 1919, **38**, 81). Estimate creatinine in the filtrate as described above.

Tissues—Mince finely and mix with a known volume of water. Mix by shaking, filter, and treat the clear solution in the same manner as a plasma sample. For the estimation of total creatinine in tissue, autoclave the suspension of the material in water at 30 lb. pressure for 1 hr. and treat as above.

Results—Recovery experiments in which known amounts of creatinine were added to human and dog plasma showed that the zinc hydroxide gave rather better recoveries than the tungstate method. Creatine, ammonia, guanidine, methyl guanidine, sarcosine, and uric acid gave no reaction with the reagents and the hydantoin reaction was so slight as to make the reagent almost specific for creatinine in biological fluids. The specificity of the reagent was compared with that of the Folin method by estimating the apparent creatinine content of autoclaved rat tissue before and after treatment with a specific creatinine-destroying bacillus. The ratio of apparent to true creatinine was consistently smaller with this method than with the Folin method. The results for true creatinine agreed well with those of other workers. When creatinine was estimated in blood and urine by this method the creatinine clearance gave a true measure of filtration rate. J. S. HARRISON

Micro-determination of British Anti-Lewisite. G. H. Spray (*Biochem. J.*, 1947, **41**, 360–361)—A method for the micro-determination of British anti-lewisite, 2 : 3-dimercaptopropanol (BAL), is described with its application to plasma, blood, and urine.

Method—Micro-determination in simple aqueous solution—Mix 1 ml. of 0·5 per cent. cobalt nitrate solution with 0·5 ml. of 2 per cent. gum arabic solution and sufficient 0·1 *N* borate buffer at pH 9 to bring the final volume, including the BAL, to 10 ml. Heat to 45° C. in a water-bath, add the BAL solution, set aside at 45° C. for 10 min., and measure the colour intensity with a Pulfrich photometer and light of wavelength 4700 A. There is linear proportionality between colour intensity and BAL concentration in the range 10 to 200 μg. A precipitate forms if the solutions are heated to a temperature higher than 45° C. Acetic acid is added to stabilise the BAL in biological fluids and this causes a slight fall in colour intensity. Physiological concentrations of sodium chloride do not interfere with the colour development. Recoveries of 96 to 102 per cent. are obtained with aqueous solutions of known strength.

Selectivity of the method—Other thiols give colours with cobalt nitrate under the conditions described, and although the colours are often indistinguishable by the naked eye from the colour given by BAL the points of maximum light absorption are usually slightly different from that of the BAL colour. The intensities of the colours, calculated on a basis of SH content, vary widely.

Application of the method to plasma, blood, and urine—Added BAL could not be recovered quantitatively from plasma, blood or urine, probably owing to destruction of part of the BAL as a result of the highly reactive nature of the 1 : 2-dithiols, and not to any defect of the method. Recoveries varied from 30 to 88 per cent.

J. S. HARRISON

Microbiological Assay of Subtilin. J. C. Lewis, E. M. Humphreys, P. A. Thompson, K. P. Dimick, R. G. Benedict, A. F. Langlykke, and H. D. Lightbody (*Arch. Biochem.*, 1947, **14**, 437–450)—The turbidimetric bacteriostatic method described is based on McMahan's assay for penicillin (*J. Biol. Chem.*, 1944, **153**, 249).

METHOD—*Cultures and inoculum*—*Micrococcus conglomeratus* (MY), *Staphylococcus aureus* (H), and, occasionally, *Streptococcus faecalis* (N.R.R.L. B–537, A.T.C.C. 7080) are used as test organisms. Maintain cultures on Schmidt and Moyer's medium II (*J. Bact.*, 1944, **47**, 199) containing 0·5 per cent. of peptone, 0·15 per cent. of yeast extract, 0·15 per cent. of beef extract, 0·35 per cent. of glucose monohydrate, and 0·4 per cent. of potassium hydroxide with the addition of agar. Transfer weekly, incubate at 37° C., and store in the refrigerator. For the inoculum, transfer a loop of cells from a slant to 150 ml. of liquid medium and incubate overnight at 37° C. To the medium add 4 per cent. of the suspensions when *M. conglomeratus* and *S. faecalis* are used, and 2·5 per cent. for *S. aureus* in the final assay cultures.

Assay medium—For *S. aureus* and *S. faecalis* use medium II above. For *M. conglomeratus* use a medium containing 2 per cent. of trypsin-digested casein and 0·5 per cent. of yeast extract with the same amounts of glucose and potassium dihydrogen phosphate as medium II. Prepare the medium double strength to allow for additions. Cool to refrigerator temperature before use.

Subtilin standard—An arbitrarily chosen, partially purified lot of subtilin was chosen as standard. This was isolated by a method described by Dimick *et al.* (*Arch. Biochem.*, in press). It was stored in the refrigerator. A solution containing 40 μg. per ml. and adjusted to pH 2·5 with hydrochloric acid was stored in the refrigerator for routine use. It was renewed every 2 to 3 months.

Samples—Dilute aqueous culture samples with 3 volumes of 95 per cent. ethanol and shake for at least 1 hr. before use. Dilute butanol extracts with 70 per cent. ethanol. A concentration of 0·5 per cent. of ethanol in the final assay culture has little effect on the response. A 1 : 1 mixture of ethanol and butanol can only be tolerated at 0·1 per cent. or lower. Dissolve lyophilised subtilin preparations in dilute hydrochloric acid of pH 2·5. Triturate insoluble fractions with glacial acetic acid. If, on dilution, these give colloidal suspensions, dilute to about 40 μg. of activity per ml. with dilute hydrochloric acid at pH 2·5 and rock gently overnight in the cool room. Unneutralised acetic acid interferes at 0·05 per cent. concentration in the final assay culture. Store samples in the refrigerator pending assay.

Procedure—Use unsterilised, 18 × 150-mm. Pyrex culture tubes with 15 ml. of total medium. Measure triplicate aliquots of standard and sample dissolved in dilute hydrochloric acid at pH 2·5 to give a volume of 5 ml. Add 10-ml. portions of cold, inoculated medium in essentially the same order as that for the subtilin solutions. Also prepare tubes with no subtilin and with sufficient to give complete inhibition of growth. Immediately incubate in a water-bath at 37° C. without disturbance for 4 hr. with *S. aureus* and *S. faecalis* and for 5 hr. with *M. conglomeratus*. After incubation cover the tubes with cotton-lined, stainless steel covers and sterilise under steam pressure. Measure the turbidity on the following day in an absorptiometer with a red filter. If readings are required on the same day steam at atmospheric pressure, otherwise there are marked changes in turbidity during the reading period.

Calculations—Convert the readings to a percentage of the maximum turbidity obtained with control not containing subtilin and plot the standard response on probability paper. This gives approximately linear standard curves for *M. conglomeratus* and *S. aureus*. An approximately linear curve for *S. faecalis* is obtained with logarithmic probability paper.

Considerable trouble was experienced in maintaining constancy of response to the standard throughout the assay experiments. To correct for this the standard solution was inserted in several positions throughout the assay. Progressive shifts in the activity of the standard as large as 15 per cent. were frequently observed in experiments with about 20 samples. An arbitrary standard curve, approximating the mean curve, was drawn and the apparent activities of the standard as well as the unknowns were estimated. The assays of unknowns were then corrected proportionately to the apparent activities of the adjacent standards.

Additional evidence is presented that the antibiotic activity of crude culture extracts of *Bacillus subtilis* and of partially purified lots of subtilin is not homogeneous. The activity of subtilin under assay conditions is unaffected by various compounds, including the common amino acids and the vitamins of the B group. J. S. HARRISON

Re-examination of Halibut-Liver Oil. Relation between Biological Potency and Ultra-Violet Absorption due to Vitamin A. R. A. Morton and A. L. Stubbs (*Biochem. J.*, 1947, **41**, 525–529)—A re-examination of the two halibut-liver oils used in the co-operative assays of 1936 has been carried out by photo-electric spectrophotometry. In 1936 it was calculated that the estimated biological activity of the mixed oils expressed in i.u. per g. divided by the intensity of absorption at 328 mμ. expressed in terms of $E_{1\,\text{cm.}}^{1\%}$ was 1570. At that time it was believed that if the absorption curve obtained for a solution of a rich oil was the same as that obtained for an equivalent solution of its unsaponifiable fraction there was no irrelevant absorption. It is now shown that the belief is not usually justified. In a later co-operative experiment a solution of vitamin A β-naphthoate gave a conversion factor of 1770 and a similar assay with cod-liver oil gave 1820. A re-examination of the two samples of halibut-liver oil has been carried out to explain the discrepancy between the factors of 1570 and 1770.

The oils were dissolved in *cyclo*hexane and their absorption curves determined by means of a Beckman photo-electric spectrophotometer. The antimony trichloride colour test was carried out by measuring intensities of absorption at 617 and 583 mμ. with a Hilger-Nutting visual spectrophotometer. Using the method of Morton and Stubbs (*Analyst*, 1946, **71**, 348), the irrelevant absorption at 328 mμ. was estimated and the corrected intensities of absorption were calculated. From the known complete absorption curve for vitamin A and the observed values for the two oils, complete subtraction curves for irrelevant absorptions were constructed. From the irrelevant absorptions so obtained a corrected value of $E_{1\,\text{cm.}}^{1\%}$ 328 mμ. for the original mixed oils was calculated, and from this a conversion factor of 1830 was obtained; this falls in line with the value of 1770 obtained from the assays on crystalline vitamin A β-naphthoate and 1820 obtained using cod-liver oil.

The results of fractionation experiments on the oils support the method of correction for irrelevant absorption. The significance of the results in the spectrophotometric assay of vitamin A is discussed. J. S. HARRISON

Chemical Estimation of the Oestrogens in Urine. A. E. Bender and A. Wilson (*Biochem. J.*, 1947, **41**, 423–425)—The method of Talbot *et al.* (*J. Biol. Chem.*, 1940, **134**, 319) for the chemical estimation of oestradiol in normal urine is compared with the biological assay. The method consists essentially of the isolation of the weakly phenolic ketones in urine, coupling with diazotised dianisidine, and measurement of the colour produced. Analyses were carried out on human urine, with and without added oestrone and on rat urine before and after ovariectomy. The results show that Talbot's method gives figures many times higher than those obtained by the biological method. The values obtained for the urine of totally ovariectomised animals were not lower than those for normal animals, which indicates that it is not oestrogenic material that is producing the colour reaction. No chemical method is thus available for estimating oestrogens in normal urine, and the biological assay remains the only trustworthy method. J. S. HARRISON

Estimation of Oestrogens in Human Pregnancy Urine. New Method of Correcting for the Brown Colour Developed in the Kober Reaction by Non-Oestrogenic Substances. M. F. Stevenson and G. F. Marrian (*Biochem. J.*, 1947, **41**, 507–511)—The determination of oestrogens in human pregnancy urine by the Kober method (*Biochem. Z.*, 1931, **239**, 209) is complicated by the fact that urinary oestrogen concentrates may contain substances that yield a brown colour in the reaction with the phenolsulphonic acid reagent. A simple procedure for correcting for the non-oestrogen brown colour has been worked out.

Procedure—Hydrolysis and extraction of urine—Collect urine samples and add toluene as preservative. Dilute 24-hr. specimens to a volume of 2·5 litres. Heat 100 ml. of the diluted urine to boiling-point under a reflux, add 15 ml. of concentrated hydrochloric acid down the condenser, and continue boiling for 30 min. Cool rapidly, extract once with 100-ml. and twice with 50-ml. portions of ether. Wash the combined ethereal extracts three times with 25-ml. portions of 5 per cent. sodium bicarbonate solution and back-extract the combined washings once with 20 ml. of ether. Warm the residue with about 3 ml. of ethanol and add 100 ml. of benzene. Extract the benzene solution once with 50 ml. and twice with 25-ml. portions of *N* sodium hydroxide. Acidify the combined alkaline extracts with 15 ml. of concentrated hydrochloric acid and extract once with 100 ml. and twice with 50-ml. portions of ether. Wash the ethereal extracts twice with 20-ml. portions of 5 per cent. sodium bicarbonate solution and back-extract the washings with 20 ml. of ether. Wash the combined ethereal extracts three times with 20-ml. portions of water and evaporate to dryness. Dissolve the residue, consisting of the total ether-soluble phenolic fraction of the hydrolysed urine, in ethanol and pipette out suitable samples for the colorimetric assay.

Technique of the Kober reaction—Prepare the phenolsulphonic acid reagent according to the method of Cohen and Marrian (*Biochem. J.*, 1934, **28**, 1603). The technique of colour development is essentially that of Venning *et al.* (*J. Biol. Chem.*, 1937, **120**, 225). Evaporate to dryness in a stream of air measured samples of the solution of urinary phenolic fraction containing between 10 and 80 μg. of oestrogen, and equivalent to not more than 2 per cent. of the 24-hr. urine specimen, contained in test tubes of 2 cm. diameter and bearing graduation marks at 8 and 15 ml. To the residues add 3-ml. portions of the Kober reagent and heat in a boiling water-bath for 20 min. Cool in an ice-salt freezing mixture, add 3 ml. of water to each tube, and mix thoroughly. Heat the tubes for 3 min. in boiling water, cool to room temperature by immersing in water, and dilute to 15 ml. with 10 per cent. v/v sulphuric acid. Measure the intensities of absorption at 520 mμ. on 7-ml. portions of the final solutions in a Spekker photo-electric absorptiometer, using an Ilford spectrum green No. 604 light filter. Heat the 8-ml. portions of the solutions remaining in the tubes in boiling water for 1·5 hr. in order to cause the pink colour produced by the oestrogen to fade. Cool, make good the water lost by evaporation, and again measure the absorption at 520 mμ. The amount of oestrogen as oestriol originally present in each tube is obtained by referring the difference between the initial and final absorptiometer readings to an oestriol calibration curve. The brown solutions yielded by ether-soluble phenolic fractions of human male urine in the Kober reaction undergo little change, as judged by absorption at 520 mμ., when heated at 100° C. for 1·5 hr.

Recovery experiments in which oestrone and oestriol were added to hydrolysed human male urine showed that when less than 10 mg. of oestrogen per 24 hr. was present, necessitating the carrying out of the colour reaction on the equivalent of 1 per cent. or more of the 24 hr. urine specimen, the recoveries obtained by the uncorrected Kober method were grossly high, whereas those obtained by the fading technique were mostly within the range 80 to 110 per cent. When the oestrogen per 24 hr. was more than 15 mg., a correction was hardly necessary. J. S. Harrison

Agricultural

Fluorimetric Method for Estimating Small Amounts of Chlorophyll *a*. R. H. Goodwin (*Anal. Chem.*, 1947, **19**, 789–794)—Purified chlorophylls *a* and *b* were prepared by the following procedure (Zscheile *et al.*, *Bot. Gaz.*, 1941, **102**, 463; 1934, **95**, 529). Grind 800 g. of fresh spinach leaves in 100-g. portions in a mortar with cold acetone, filter each extract, and transfer the pigment from each batch to light petroleum. Wash the petroleum solution with water, methyl alcohol, and again with water until the chlorophyll is precipitated. Dry the suspension over anhydrous sodium sulphate, collect the precipitate on a 3-cm. layer of powdered sucrose, wash it with light petroleum to remove carotenoids, and extract it from the sucrose with ether. Adjust the crude chlorophyll solution to contain 70 per cent. of light petroleum and 30 per cent. of ether and adsorb the chlorophyll on a sucrose column of diameter 5 cm. and length 35 cm. Develop the chromatogram for about 1·5 hr., adding during this time 500 ml. of fresh mixed solvent. Remove mechanically from opposite ends of the column the central portion of the upper green zone and the lower three-quarters of the lower blue zone and elute the pigment from each separately with ether. Wash the blue ether solution of chlorophyll *a* thoroughly with water, dry it over sodium sulphate, filter, and store it on solid carbon dioxide until immediately before spectroscopical examination.

To purify the green fraction adjust the ether eluate to the 70/30 ratio (*supra*) and re-adsorb the pigment on another sucrose column. After 2 hr. remove the central portion of the green zone and elute the pigment. Repeat the process once more with a third column. If spectroscopical examination of the eluate reveals the presence of chlorophyll *a* (as may occur in a very humid atmosphere), store the eluate in ether on solid carbon dioxide and develop a fourth column 3 days later. Elute the green band with ether, wash, dry and filter the eluate, dilute to volume and store it on solid carbon dioxide as with the chlorophyll *a* preparation. Conduct all these experiments in dim light.

Evaporate aliquots of the preparations in tared volumetric flasks in a vacuum desiccator, dry for 1 hr. at 103° C., cool, and weigh. Meanwhile make fluorimetric determinations with other portions of the solutions. Obtain absorption spectra (an automatic recording spectrophotometer, as described by Hardy, *J. Optical Soc. Amer.*, 1935, **25**, 305, is recommended) with a band-width of 10 mμ. and calculate the specific absorption coefficients (α) for chlorophylls *a* and *b* thus, $\alpha = (\log_{10}I_0/I)/cx$, where c is the concentration of the chlorophyll in g. per litre and x is the cell-thickness in centimetres.

For fluorimetric determinations, a Klett fluorimetric colorimeter is suitable (Kavanagh, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 108) with a Leeds and Northrupp Type R galvanometer. The unknown solutions of chlorophyll should be in pure acetone, and the standard fluorescent solutions, shielded by a 5970 lamp filter, may contain 1 or 0·2 mg. of quinine sulphate per litre of 0·1 *N* sulphuric acid. Measurements are made at room temperature (22° to 26° C.). Although chlorophyll fluorescence decreases with rise of temperature (Zscheile *et al.*, *J. Phys. Chem.*, 1943, **47**, 623) errors due to temperature fluctuation within this range are less than 3 per cent. Readings should be made as rapidly as possible to minimise photo-decomposition. Decay of fluorescence is most pronounced when filters transmitting ultra-violet light are used, but is negligible with blue and violet filters. Fluorescence, as measured by potentiometric readings, is proportional to the chlorophyll present at low concentrations.

Chlorophyll *a* is much more fluorescent than chlorophyll *b*; it may also be somewhat less stable on storage in ether in the dark at 0° C. The difference between the fluorescence of *a* and *b* is greatest when the fluorescence is excited by the violet line 404·7 mμ. Experiments showed that the two chlorophylls fluoresce independently, and it is thus possible to compute the fluorescence of various mixtures containing a constant total amount of chlorophyll. Pure chlorophyll *a* can be distinguished from chlorophyll *b* by the ratio $R = F_{404\cdot7}/F_{435\cdot8}$, a ratio that can be determined with considerable precision even when the concentration of the preparation is unknown, since the relative fluorescence at these wavelengths is the determining factor. For a mixture of the two chlorophylls the following relation should hold

$$R = \frac{Fa_{404\cdot7}(X)}{Fa_{435\cdot8}(X)} + \frac{Fb_{404\cdot7}(1-X)}{Fb_{435\cdot8}(1-X)},$$

where *Fa* is the fluorescence of a given concentration of chlorophyll *a*, and *Fb* the fluorescence of an equal concentration of chlorophyll *b* when excited by light of the wavelength indicated, and *X* is the proportion by weight of chlorophyll *a* in the mixture. An estimate of the proportion of *a* and *b* in an unknown mixture can be made by determining *R* and finding this value on a calibration curve showing the relation between the value of *R* and the composition of the mixture of *a* and *b*. The curve is rather flat for high percentages of chlorophyll *a* and the accuracy of the estimate is therefore low in this portion of the curve. Spectrophotometric methods (Comar, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 877; Griffiths *et al.*, *Ibid.*, 1944, **16**, 438) for obtaining the *a*/*b* ratio are preferable when the quantity of material permits. Since values of *R* for chlorophylls *a* and *b* will vary somewhat with the filter transmission, the spectral sensitivity of the photo-cells and other experimental conditions, a calibration curve must be computed for each new arrangement. This method is not applicable to crude extracts since the value of *R* is altered somewhat by the presence of other pigments. However, chromatographic separations of chlorophylls from the carotenoids can be carried out on small amounts of material (Goodwin *et al.*, *Plant Physiol.*, 1947, **22**, 197).

Estimation of chlorophyll a—Since chlorophyll *a* fluoresces about ten times as much as chlorophyll *b* when excited by the line 404·7 mμ., the assumption can be made that all the fluorescence emitted by mixtures of the two components under these conditions is due to the fluorescence of *a*. For extracts of green tissues usually containing about 70 per cent. of chlorophyll *a*, the theoretical error should be about 4 per cent. If enough material is available a determination of the ratio *a*/*b* may be made either fluorimetrically or spectrophotometrically. Then, assuming constancy of the ratio for subsequent samples, the chlorophyll estimate can be corrected and a computation of the total chlorophyll content of the extract can also be made.

If only the relative concentrations are required and if the ratio *a*/*b* can be assumed to remain nearly constant among the samples to be tested, greater sensitivity will be attained by using ultra-violet light or blue light as excitants. With ultra-violet light, however, readings must be made as quickly as possible to minimise photo-decomposition, particularly of the more labile chlorophyll *a* component.

In mixtures of chlorophyll *a* with β-carotene the fluorescence of the chlorophyll is least affected by the presence of β-carotene when the solution is irradiated with ultra-violet light and most affected when irradiated with violet light. The reduction in fluorescence due to carotene becomes progressively more pronounced the higher is the concentration, but at low chlorophyll concentration the carotene does not appreciably reduce the fluorescence even when present in larger amounts than chlorophyll *a*, and for appreciable interference in dilute solution of chlorophyll the carotenoids must occur in concentrations considerably higher than that found in normal green tissues. Extracts of the shoots of dark grown oat seedlings containing flavones in amount equivalent to 100-mg. fresh weight of tissue when added to chlorophyll *a* solutions cause no decrease in fluorescence. Crude extracts of certain plant material, however, may contain sufficient amounts of flavones or other pigments to interfere.

Determination of chlorophyll a in crude extracts—Weigh the fresh tissue to be extracted on a micro-balance to the nearest 0·1 mg., dip it for 30 sec. in boiling water and grind it finely with acetone in a suitable homogeniser. Centrifuge immediately and decant the clear supernatant liquid. Dilute the crude extract to 20 ml. with acetone. Determine the fluorescence of the extract or a dilution thereof immediately in a fluorimeter, using the 404·7 mμ. line, and estimate the amount of chlorophyll *a* present by means of a calibration curve.

For details of the construction and manipulation of the instruments used in this investigation the original paper should be consulted. A. O. JONES

Gas Analysis

Determination of Acetylene in Gas. J. A. Shaw and E. Fisher (*Anal. Chem.*, 1948, **20**, 533–536)—The gas is scrubbed with a 35 per cent. solution of silver nitrate, usually containing ferric nitrate. After diluting, filtering, washing, and drying, the $Ag_2C_2.AgNO_3$ formed is weighed.

Procedure—Pass a measured amount of gas at a rate of 0·5 cu. ft. per hr. successively through solutions of potassium hydroxide (20 to 30 per cent.), monochlorobenzene containing 5 to 10 g. of piperidine per litre, 10 per cent. sulphuric acid, and silver nitrate - ferric nitrate (twice). The last solution is made by dissolving 540 g. of silver nitrate in 1 litre of 3 *N* nitric acid with gentle heating and then dissolving the equivalent of 60 g. of anhydrous ferric nitrate in the cooled solution. Pass sufficient gas through to yield 100 to 150 mg. of $Ag_2C_2.AgNO_3$, and not more in view of explosion hazards. Use goggles and tongs in handling the dry precipitate and always dissolve residues in dilute hydrochloric acid. Dilute the contents of the two tubes to 300 ml., filter through a sintered-glass filter, wash with acetone, and complete the drying in a desiccator containing sulphuric acid.

The test was normally employed on coke-oven gas containing about 0·05 per cent. of acetylene, when about 0·5 cu. ft. were used. Other gases may need modifications in procedure. For example, if there is no hydrogen present, the acid ferric nitrate, which prevents the formation of metallic silver, is not necessary. The method is said to differentiate between acetylene and alkyl acetylenes.

W. J. GOODERHAM

Organic

Determination of Small Amounts of Ethyl Ether in Ethyl Alcohol. W. E. Shaefer (*Anal. Chem.*, 1948, **20**, 651–652)—*Procedure*—Transfer 500 g. (approximately 630 ml.) of sample to a 1-litre, round-bottomed flask and add 250 ml. of water and a few particles of carborundum. If the sample is suspected to contain less than 0·2 per cent. of ether, add 2 ml. of ether from a 2-ml. pipette previously rinsed with ether and cooled by a current of air. Such a prepared pipette will deliver 1·38 g. of ether and will increase the ether content of the sample by 0·28 per cent. Attach a 3-bulb Snyder column bearing a thermometer to the flask, and weigh the unit and sample correct to 0·1 g. Connect the outlet of the column to a vertically arranged condenser and attach the latter to a suitable receiver.

Direct a strong jet of air against the base of the column and heat the sample rapidly, by means of an electric heater with a variable control, to incipient boiling. Adjust the heater until the condensed vapour just refluxes from the lowest bulb of the column. Re-adjust the heater and allow the distillation to proceed slowly (approximately 0·5 ml. in 5 min.) until the vapour temperature reaches 52° C. Maintain the vapour temperature at 52° ± 2° C. until the distillation rate falls below 3 drops per min., and then heat for an additional 10 min. at 52° C. If the initial rate of distillation never exceeds 3 drops per min. in the temperature range 52° to 54° C., continue the distillation within this range for 20 min. from the time the vapour temperature first reached 52° C. Remove the heater, disconnect the condenser, and weigh the system correct to 0·1 g.

The distillation rate is controlled by adjustment of either the heater or the air jet.

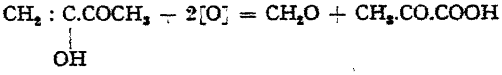
Calculation—If no ether was added before distillation, the ether content of the sample is calculated as follows:—loss in weight of sample × 100/weight of sample of alcohol = per cent. of ether (uncorrected). Apply the correction value read from a curve constructed from data obtained by distillation of 500-g. aliquots of ethyl alcohol to which known amounts of ether have been added, the percentage of ether added being plotted on one axis and the percentage found on the other.

If 2·0 ml. of ether were added to the sample before distillation, the ether content is calculated and corrected as described above and from the result so obtained 0·28 per cent. is deducted to give the ether content of the original sample.

Water, and up to 0·5 per cent. of benzene, do not interfere.

A. H. A. ABBOTT

Spectrophotometric Determination of Diacetyl. J. C. Speck, jun. (*Anal. Chem.*, 1948, **20**, 647–648)—The purple colour formed by diacetyl with chromotropic acid and sulphuric acid is nearly indistinguishable from that formed by formaldehyde under the same conditions, and is probably due to the production of formaldehyde from diacetyl by oxidation with sulphuric acid according to the scheme



$$\underset{\textstyle OH}{CH_2 : C}.COCH_3 + 2[O] = CH_2O + CH_3.CO.COOH$$

The formation of this colour has been utilised as the basis of a rapid spectrophotometric determination of diacetyl. Formaldehyde, methyl ethyl ketone, and all substances that interfere in the determination of formaldehyde with chromotropic acid, interfere with the proposed method. Acetyl propionyl, the next higher homologue of diacetyl, gives no colour with chromotropic acid.

Procedure—Transfer 1 ml. of a solution containing 0·03 to 0·1 mg. of diacetyl to a test tube provided with a glass stopper, add 0·5 ml. of 10 per cent. chromotropic acid solution, mix well, and gradually add 5 ml. of 98 per cent. sulphuric acid. Immerse the stoppered tube in a water-bath so that the level of the contents is below the water level and heat at 100° C. for 1 hr. Cool, dilute the mixture to 50 ml. with water, and determine the extinction at 570 mμ. Carry out a blank determination on the reagents and deduce the diacetyl content of the sample by reference to a standard curve constructed from data obtained by application of the above procedure to samples containing known amounts of diacetyl.

A. H. A. ABBOTT

Polarographic Determination of Methacrylic Ester. M. B. Neyman and M. A. Shubenko (*Zavod. Lab.*, 1948, **14**, 394–396)—Methyl methacrylate can be determined polarographically in aqueous alcoholic solutions containing lithium chloride or tetramethyl ammonium iodide as supporting electrolyte. The method can be used for studying the polymerisation of the ester.

In 0·1 *N* lithium chloride in 25 per cent. alcohol at 20° C. well-defined waves were obtained for methyl methacrylate in concentrations of 8, 16, and 24 mg.-mol. per litre, and the wave-heights were proportional to concentration. The reduction potential and the half-wave potential were $-1{\cdot}7$ and $-1{\cdot}92$ v., respectively, *versus* the normal calomel electrode. Calculations from the Ilkovic equation gave the diffusion coefficient $D = 3{\cdot}8 \times 10^{-6}$ cm.^{2} per sec.

Slightly better waves were obtained in 0·1 *N* tetramethyl ammonium iodide because of the more negative reduction potential of the supporting electrolyte. The diffusion coefficient is $2{\cdot}5 \times 10^{-6}$ cm.^{2} per sec. G. S. Smith

Amperometric Micro-titration of Diamidines. J. B. Conn (*Anal. Chem.*, 1948, **20**, 585–586)—Owing to the low degree of accuracy of the available methods of determining diamidines, an accurate amperometric method has been developed. It depends on the formation of the insoluble alizarin-sulphonates of the diamidines. Since alizarin-sulphonic acid is polarographically reducible, any excess is readily detected after the end-point has been reached by observing the current flowing between a dropping mercury cathode and a saturated calomel reference electrode immersed in the solution.

Procedure—Dissolve 6 to 10 mg. of the diamidine salt in 10 ml. of 0·1 *M* phosphate or borate buffer at pH 7·0, and transfer a 5-ml. portion of this solution to a polarographic cell. Pass nitrogen through the cell for 5 min. to remove dissolved oxygen and, after setting the applied potential at $-0{\cdot}9$ v. *versus* the saturated calomel electrode, run in from a burette an approximately 0·08 *M* solution of recrystallised sodium alizarin sulphonate, dissolved in the buffer at pH 7·0. At approximately every 0·02-ml. addition pass nitrogen through the solution for 2 min. and then read the current, and plot it against the volume added. Continue this until the current is increasing linearly, and then read off the end-point at the intersection of the two straight lines obtained.

The results are accurate to within ±0·5 per cent. With phenamidine, the separation of the crystalline alizarin-sulphonate occurs slowly, and longer intervals must be allowed between additions of reagent; it is helpful to add a little solid phenamidine alizarin-sulphonate at the start of the titration to provide a seed for the crystals. J. G. Waller

Determination of Lignin in Plant Material of High Protein Content. E. R. Armitage, R. de B. Ashworth, and W. S. Ferguson (*J. Soc. Chem. Ind.*, 1948, **67**, 241–243)—Extract 1 g. of ground (1/64-in. mesh) sample in a boiling mixture of alcohol and benzene (1 : 2) in a sintered-glass crucible placed in a fat-extraction apparatus, wash the residue with ether, and dry it by suction. Heat it under reflux for 1 hr. with 100 ml. of 5·0 per cent. (w/w) hydrochloric acid, filter it off through a fine cloth, wash it with water, and digest it with 100 ml. of 0·25 per cent. sodium carbonate solution and 0·1 g. of trypsin for 18 hr. at 38° C. Filter through cloth, wash the residue with alcohol and ether, dry it by suction, and stir it with 10 ml. of 72 per cent. sulphuric acid solution until no lumps remain. After 2 hr. at 15 to 20° C., transfer it to a 500-ml. flask with 240 ml. of water, and heat the mixture under reflux for 2 hr. Filter while hot through a tared asbestos Gooch crucible (alundum is untrustworthy), keeping the crucible full to accelerate filtration. Wash the residue well with hot water, dry it in a steam-oven until constant in weight, and weigh it quickly. Burn off the lignin in a furnace, and obtain its weight by difference, after reweighing the crucible. Make the determination in triplicate, treating two samples as above, but filtering the third through filter-cloth (instead of on a Gooch crucible) and then washing and drying it. Determine the nitrogen content and deduct this × 6·25 from the mean crude lignin content as obtained from the two other experiments. Data tabulated for grass, hay, oat straw, clover, and sheep's faeces show the effect of variations in the method of pre-extraction with solvent, enzyme treatment, protein content, and concentration of the acid hydrolysis. Comparison with the methods of Norman, of Crampton and Maynard, and of Davis and Miller show that the new method gives much lower results. They are, however, regarded as much nearer the true figure because the nitrogen correction is much less (2·0 per cent.), and because the treatment prior to acid hydrolysis removes most of the substances likely to form insoluble complexes with the strong acid used. J. Grant

Measurement of Enzymic Activity on Limit Dextrin. T. M. Back, W. H. Stark, and R. E. Scalf (*Anal. Chem.*, 1948, **20**, 56–60)—When corn (maize) starch is treated with malt enzymes, 65 to 75 per cent. is converted to fermentable sugars in 15 min. The rate of reaction then decreases and the residual amylase-resistant "limit dextrin" is only slowly converted to fermentable sugar. The efficacy of enzymic preparations for the conversion of diastatically produced limit dextrin to fermentable sugar is of great technical importance, particularly when rapid and complete conversion is desired.

The method presented was devised to evaluate enzymic preparations on the basis of their power to convert diastatically produced limit dextrin to fermentable sugars (*i.e.*, their dextrinase activity) under standard conditions of time, temperature, pH, and substrate concentration. It is based on the difference in reducing power (after acid hydrolysis) in the blank medium (in which enzyme has been destroyed before limit dextrin has been added) and the enzyme-treated sample after complete, rapid removal of fermentable sugar from a dilute solution by a large amount of baker's yeast. A

2·5-hr. treatment of the dilute solution of sugars at pH 4·8 and at 30° C. with fresh baker's yeast (15 g. per 100 ml. of liquid) was selected. These conditions have been shown by Stark and Somogyi (*J. Biol. Chem.*, **1942**, **142**, 579) to result in complete removal of maltose. If the fermentable sugar consists only of glucose, the amount of yeast may be reduced to 5 g. per 100 ml. and the fermentation period to 1 hr.

Since unwashed yeast contains a variable amount of reducing substances, Somogyi's method (*Ibid.*, **1927**, **75**, 35; *Analyst*, **1927**, **52**, 719) of repeatedly washing and centrifuging the yeast until the wash water remains clear was adopted. The slight dilution of the sugar solution that occurs when the washed yeast is added may be corrected for by determining the reducing power of the blank before and after yeast sorption.

Reducing power before and after removal of fermentable sugars was determined by the Shaffer - Hartmann micro-method (Shaffer *et al.*, *Ibid.*, 1921, **45**, 379) as described by Stiles *et al.* (*J. Bact.*, 1926, **12**, 429). The proposed method for the determination of limit dextrinase activity (mg. of fermentable sugar produced from limit dextrin by 1 g. of enzyme) is based on the fact that conversion of a standard solution of limit dextrin is proportional to the amount of enzyme employed up to a certain degree of conversion. This degree of conversion must be determined experimentally for the particular enzyme used, for it is essential that the reaction should occur within the range where a linear relation exists between the conversion and the amount of enzyme used.

To prepare diastatically produced limit dextrin, first prepare malt extract for use as "pre-malt" and as the saccharifying agent by extracting 550 g. of finely ground barley malt with 2 litres of water for 1 hr. with frequent shaking followed by centrifuging. To 12 litres of water add 1·8 kg. of corn starch dispersed in water, warm to 68° C., and add 320 ml. of the malt extract, also at 68° C. Agitate the dispersion at 80° C. for 2 hr. and then pressure-cook at 60 lb. per sq. in. for 7·5 min. (or at 15 to 20 lb. per sq. in. for 1 hr.). Cool to 58° C., adjust the pH to 5·5, and add 1280 ml. of the malt extract. After hydrolysis for 45 min. at 55° to 58° C., autoclave the hydrolysate for 1 hr. at about 16 lb. per sq. in. to destroy the enzymes. Cool to 30° C., add 123 g. of baker's yeast, adjust the volume to 16 litres, and maintain at 30° C. for 44 hr. Centrifuge to remove solid matter, reduce the volume to about 1 litre by evaporation under reduced pressure, filter the concentrate, and add the filtrate to 2 to 4 times its volume of methyl alcohol, stir, and allow to stand overnight. Remove the gummy residue from the main bulk and purify it by re-dissolving it in a small amount of water, filtering, and re-precipitating it with methyl alcohol. Wash it repeatedly with methyl alcohol and dry it in a vacuum oven at 50° C. Finally, powder the dried limit dextrin and pass it through a fine sieve.

The buffer solution is an aqueous solution of 35·32 g. of disodium phosphate dodecahydrate and 9·73 g. of citric acid diluted to 1 litre, the pH being adjusted, if necessary, to 4·8 by addition of either component. To prepare washed yeast suspend baker's yeast repeatedly in fresh portions of water and centrifuge until the wash water remains clear. After the final wash water has been decanted press the yeast on absorbent paper.

If dry enzymic preparation is to be tested, first grind it thoroughly and then extract 0·9 g. with exactly 20 ml. of water for 1 hr. at 30° C. in a tightly stoppered flask with continuous agitation. After centrifuging, the decanted extract is ready to be diluted (*infra*). With liquid preparations extraction is unnecessary and the material is simply centrifuged or filtered through glass wool.

Procedure for determining limit dextrinase activity—To determine the range in which limit dextrin conversion is linear with respect to enzyme concentration it is necessary to determine the conversion produced with a series of different amounts of the enzyme. To do this add 5-ml. portions of diluted enzyme extract, the stock enzyme extract (*supra*) being diluted to give a series of enzyme extracts containing varying amounts of the stock extract, and then plot the degree of conversion of limit dextrin against the amount of enzyme used.

Place the required number of 50-ml. flasks, each containing a 20-ml. portion of limit dextrin solution containing 0·18 g., in a water-bath at 30° C., allow the contents to attain this temperature, and to each flask add 5 ml. of enzyme extract of the appropriate dilution to give an enzyme concentration within the linear range. Allow the action to proceed for 60 min., and then destroy the enzyme by adding 5 ml. of 1·5 *N* sodium hydroxide. After 30 min. adjust the pH to 4·8 with 1·5 *N* sulphuric acid, using methyl red as indicator. If the enzymic agent is buffered, the pH of the conversion mixture should be determined, as it is necessary that the conversion should be made at pH 4·8. It is desirable to determine limit dextrinase action with at least three different enzyme concentrations, but concentrations below 3 per cent. should be avoided.

For the blank determination place 5 ml. of the enzyme extract in a 50-ml. flask containing 5 ml. of 1·5 *N* sodium hydroxide. After 30 min. add 20 ml. of the standard limit dextrin solution and adjust the pH to 4·8 with 1·5 *N* sulphuric acid, using methyl red as indicator. Then treat the blank exactly as described for the samples. (If the enzymic agent contains no fermentable or reducing substance after acid hydrolysis, the blank can be run in exactly the same manner as the samples, except that no enzyme extract is added.)

After the samples and the blank have been adjusted to volume, place a 20-ml. portion from each in 40-ml. graduated centrifuge tubes. From the blank remove a second 10-ml. portion and add it to 10 ml. of 1·38 *N* hydrochloric acid. To each of the 20-ml. portions previously placed in centrifuge tubes add 3 g. (moist weight) of washed fresh baker's yeast, shake the tubes thoroughly, and maintain them at 30° C. for 2·5 hr., shaking them several times during that period. Centrifuge each tube, decant the liquid portion and add exactly 10 ml. to 10 ml. of 1·38 *N* hydrochloric acid. Hydrolyse all the samples that have been added to hydrochloric acid in boiling water for 2·5 hr., cool, neutralise to the phenolphthalein end-point with 1 to 2 N sodium hydroxide, and dilute to 100 ml. Add triplicate 5-ml. portions from each sample to 5-ml. portions of sugar reagent and determine reducing sugar as described in the standard method (Stiles *et al., loc. cit.*). In the accurate determination of reducing sugar a curve is drawn on a large scale relating a series of 5 to 50 mg. per 100-ml. samples of dextrose to the thiosulphate titration. The result should be a straight line, or nearly so, not passing through the origin.

The percentage conversion is given by $100(B_2 - G_2)/B_2$, where B_2 is mg. of glucose in a 5-ml. sample of the blank after yeast sorption, the sample being removed after acid hydrolysis and final dilution, and G_2 is mg. of glucose in a 5-ml. portion of the enzyme-treated sample after yeast sorption, the sample being removed after acid hydrolysis and final dilution. Defining limit dextrinase units as mg. of fermentable sugar produced from limit dextrin by 1 g. of enzyme preparation in 1 hr. at 30° C., the number of dextrinase units is given by $100(B_2 - G_2)/E(B_2 \div B_1)$ where B_1 is mg. of glucose in a 5-ml. portion of the blank before yeast sorption, the sample being removed after acid hydrolysis and final dilution, and E is g. of enzyme preparation extracted per 5 ml. of enzyme extract added to the 20-ml. portion of limit dextrin. The factor $(B_2 \div B_1)$ corrects for dilution due to the use of washed yeast.

By slight modifications of the standard method given, the effect of any variable on limit dextrinase activity may be studied. The method was applied to determination of the effect of the enzyme-limit dextrin ratio on conversion of limit dextrin over a wide range for Mylase bran and a 48-hr. submerged culture of *Aspergillus oryzae*. A. O. JONES

Separation and Determination of Polyols by Selective Entrainment. Application to the Determination of Glycerol. G. Métayer (*Chimie Analyt.*, 1948, **30**, 148–156)—*Apparatus*—This is a Dean and Stark apparatus with a 250-ml. flask. The receiver is of 7 to 10 ml. capacity, graduated in 0·1 ml., and is equipped with a draw-off stopcock. The apparatus is assembled with interchangeable ground joints and 14-cm. columns can be inserted between the receiver and the condenser.

Procedure—Mix 2 to 3 g. of the polyol mixture with 80 to 100 ml. of the selected entraining liquid and distil until no further increase in volume of the entrained liquid is apparent. Wash the condenser 5 or 6 times with 15 to 20 ml. of the entraining liquid, stir with a fine glass rod, and read the volume entrained.

Determination of water in polyols—Chloroform saturated with water is the recommended entrainer and for this procedure the stopcock is connected to a tube that leads the entrainer back into the flask. When employed for mixtures of ethylene glycol and water the latter is entrained quantitatively with about 5 per cent. of the glycol.

Separation of polyols with aromatic hydrocarbons—Benzene, toluene, and xylene are studied as entrainers, but the only successful separation is that of ethylene glycol from diethylene glycol and glycol by means of toluene.

Separation of polyols with cyclo*paraffins*—The behaviour of individual polyols is shown in the following table.

	Ethylene glycol	Propylene glycol	Trimethylene glycol	Diethylene glycol	Glycerol
*Cyclo*hexane	+	+	+	–	–
Methyl*cyclo*hexane	+	+	+	O	–
Dimethyl*cyclo*hexane ..	+	+	+	+	+

+ signifies quantitative entrainment, O partial entrainment, – no entrainment.

*Cyclo*hexane affords quantitative separation of ethylene glycol and/or propylene glycol from diethylene glycol and glycerol, but a 25-cm. column is necessary to prevent entrainment of diethylene glycol by ethylene glycol. The distillation requires up to 8 hr. Trimethylene glycol can be separated from diethylene glycol with *cyclo*hexane but not from mixtures containing a high proportion (70 to 80 per cent.) of glycerol unless ethylene or propylene glycol is also present to assist entrainment of the trimethylene glycol. Mixtures of trimethylene glycol and glycerol, containing less than 30 per cent. of the glycol, can be separated by entrainment with methyl*cyclo*hexane, but the distillation must not be prolonged beyond 8 hr. Decalin entrains glycerol quantitatively in 30 min. and diethylene glycol in 1 hr. It also entrains erythritol and quinitol, but not D-mannitol, D-sorbitol, inositol, or arabitol. Glucose, sorbose, and arabinose carbonise during the distillation.

In a mixture of polyols, water is determined by distillation with chloroform; ethylene, propylene, and trimethylene glycols are determined collectively by distillation of a second sample with *cyclo*hexane; total glycols and glycerol are determined by distillation of a third sample with decalin.

The polyol present, for example, in a cream, can be determined by distillation with decalin, followed by measurement of the refractive index and specific gravity of the polyol-water mixture. Gerlach's table (D. Holde, *Huiles et grasses*, 1929, 770) gives the percentages of water-glycerol mixtures in terms of the physical constants, and similar tables may be prepared for the glycols. For a table dealing with diethylene glycol, see Palfray, Sabetay, and Libmann-Métayer (*Industrie de la Parfumerie*, **2**, 325). W. C. JOHNSON

Determination of Unsaturation of Synthetic and Natural Rubbers by Means of Iodine Monochloride. T. S. Lee, I. M. Kolthoff, and M. A. Mairs (*J. Polymer Sci.*, 1948, **3**, 66–84)—The reaction of iodine monochloride with the unsaturated hydrocarbon polymers of isoprene and butadiene, and butadiene-styrene co-polymer has been investigated. Three procedures for determining the unsaturation of these polymeric materials are given. In the first procedure the reaction-rate curve is obtained in which correction is made for substitution, the flat portion of the corrected reaction-rate curve giving the true unsaturation. A second procedure is applicable only to polymers actually covered by the present investigation for which empirical corrections are available, and involves a smaller number of iodimetric determinations. A third procedure is for the routine examination of certain types of polymer and is less accurate, errors of 2 per cent. being possible owing to lack of reproducibility in the amount of substitution occurring. The methods have been applied only to uncompounded, specially purified, raw rubbers.

Nature of the reactions involved—In the presence of an excess of iodine monochloride 90 to 95 per cent. of the double bonds in polymers react within a few minutes at room temperatures and, after this time, the remaining double bonds react only very slowly. The "splitting out" of hydrohalic acid, due, almost certainly, to some form of cyclisation, also occurs within the first few minutes of the reaction period. Any acid appearing after this time is the result of substitution. The acid formed is invariably hydriodic acid, which reacts with an excess of iodine monochloride as quickly as it is formed, to give iodine and hydrochloric acid. The iodine can therefore be estimated instead of the acid. This is done by titration with 0·02 *N* (0·005 *M*) potassium iodate solution, the end-point being indicated by the disappearance of the iodine colour from the organic phase. This reaction proceeds according to the equation

$$KIO_3 + 2I_2 + 6HCl = 3H_2O + KCl + 5ICl$$

Preparation of the sample—If the polymer contains more than 0·2 per cent. of anti-oxidant or other impurities, or if it contains gel, purification by precipitation is necessary. Cut 5 g. of the polymer into small pieces and place in 500 ml. of benzene. Allow to stand until dissolved or for 2 to 3 days. Decant the clear liquid and remove any gel from the solution by filtration through glass wool or a wire screen. Precipitate by the slow addition of the solution to 2 litres of absolute alcohol containing 0·2 per cent. of phenyl-β-naphthylamine. Stir the alcohol rapidly during the addition. Separate the polymer by decantation or filtration, wash with 100 to 200 ml. of 0·2 per cent. phenyl-β-naphthylamine in alcohol, remove the excess of washing liquid by blotting with filter paper, and dry at 80° C. in a vacuum oven for 1 hr., or, with an isoprene polymer, dry at room temperature for 24 hr. at a pressure of less than 10 mm. If the polymer is obtained from a latex, use a similar procedure, coagulating each 20 ml. of latex with 1 litre of alcohol.

Procedure I—Weigh 0·25 g. of the purified polymer (0·20 g. for polybutadiene) and transfer to a 250-ml. volumetric flask. Introduce the rubber through the neck of the flask with the aid of a tube of paper to prevent adhesion of the raw rubber to the glass. Add 50 ml. of chloroform and 135 to 140 ml. of carbon disulphide. Set aside or agitate gently until dissolution is complete (12 hr. to 2 days). Add 50 ml. of 0·11 *N* iodine monochloride solution in chloroform, dilute to the mark with carbon disulphide, and mix thoroughly.

After 10 to 15 min., pipette 25 ml. of the solution into 50 ml. of 6 *N* hydrochloric acid in an iodine flask. Titrate with 0·02 *N* (0·005 *M*) potassium iodate solution, the end-point of the titration being given by the disappearance of iodine from the organic phase. After successive periods of 0·5, 1, 2, and 4 hr., determine the total iodimetric titre as well as the free iodine. For the total iodimetric titre pipette 25 ml. of the reaction mixture into potassium iodide solution containing 40 ml. of water, 10 ml. of alcohol, and 0·5 g. of potassium iodide. Shake and titrate immediately with 0·05 *N* thiosulphate solution. Add starch near the end-point. Prepare a blank reaction mixture in exactly the same way as the sample reaction mixture and titrate, except that it is only necessary to carry out one titration against iodate and one against thiosulphate. Calculate the corrected unsaturation as,

$$\text{Per cent. unsaturation} = \frac{[a - (b - c)] \times \text{M.W.}}{w}$$

where a = decrease in total iodimetric titre in mg.-mol. after 0·2 to 4 hr.
b = number of mg.-mol. of iodine found after 0·2 to 4 hr.
c = number of mg.-mol. of iodine found up to 0·2 hr.
M.W. = molecular weight of the monomer unit; *i.e.*, 54·1 for butadiene and 68·1 for isoprene polymers.
w = weight of the sample in grams.

Procedure II—This is identical with Procedure I except that the number of the titrations is less. Titrate an aliquot portion with potassium iodate after 10 min. as before and determine the total iodimetric titre and repeat the iodate titre at the end of the time periods specified as necessary for complete addition; these are given in Table I.

Procedure III—Weigh into a 300-ml. iodine flask 0·100 g. of GR-S (butadiene-styrene copolymer) or isoprene, or 0·080 g. of polybutadiene. Add 20 ml. of chloroform and 60 ml. of carbon disulphide. Set aside or agitate gently until the sample is dissolved. Add 20 ml. of 0·11 *N* iodine monochloride solution in chloroform and allow to stand for the period specified for complete addition (see Table I). After this period, add 50 ml. of water containing 0·5 g. of potassium iodide. Shake and titrate with 0·1 *N* thiosulphate. Titrate also a blank prepared at the same time.

$$\text{Per cent. unsaturation} = \left[\frac{a \times \text{M.W.}}{10 \times w}\right] - d$$

where d is the correction for substitution given in Table I, and the other symbols are as before.

Presentation of results—Percentage unsaturation as calculated is equivalent to the apparent percentage of polymerised butadiene or isoprene in the sample. To express these figures as iodine values, multiply by 4·696 for butadiene polymers or its copolymers with styrene, or by 3·728 for isoprene polymers. Procedure I gives the corrected unsaturation for a number of reaction periods, the values for which can be plotted against time on a graph. The linear part of this graph is held to give the true figure for the unsaturation. The linearity of this curve is illustrated by reference to Table II, which gives results obtained by Procedure I for several types of polymer.

TABLE I

Polymer	Temp. ° C.	Time needed for complete addition hr.	Approx. amount of substitution*
Emulsion butadiene-styrene	25	1·0	1·2
Emulsion polybutadiene	25	1·0	1·0
Emulsion polyisoprene	25	0·2	1·0
	0	0·4	1·0
Sodium butadiene-styrene	25	1·0	2·3
Sodium polybutadiene	25	1·0	1·7
Sodium polyisoprene	25	0·2	2·8
	0	0·4	2·5

* Expressed as mol. per cent. of total number of double bonds, the value given being that required for *d* in Procedure III.

TABLE II

Type of polymer	Temp. ° C.	Time hr.	Substitution %	Corrected unsaturation %
GR–S (64% conversion)	25	0·35	0	81·5
		1·08	1·6	80·9
		2·07	2·3	80·9
		4·42	3·5	80·9
Emulsion polybutadiene	25	0·17	0	95·6
		1·0	0·9	97·7
		4·0	1·6	97·7
		6·0	1·9	98·1
Emulsion polyisoprene	0	0·1	0	96·3
		1·2	1·4	96·8
		4·0	3·0	96·8
	25	0·1	0	96·6
		1·3	3·8	97·3
		4·3	6·7	97·3
Sodium polybutadiene	25	0·15	0	88·8
		1·0	1·7	92·1
		2·6	3·4	92·2
		5·0	4·6	91·9
Natural rubber	0	0·17	0	97·8
		1·0	1·7	96·7
		4·2	3·4	96·5

W. C. WAKE

Determination of the Nitrosoamine Content of Propellant Explosive Stabilised with *sym*-Diethyldiphenylurea. T. C. J. Ovenston and C. A. Parker (*J. Soc. Chem. Ind.*, 1947, **66**, 394–395)—In the approximate method of Lécorché and Jovinet (*Mem. Poudres*, 1928, **23**, 153) for the surveillance of double base propellant powder stabilised with *sym*-diethyldiphenylurea, N-nitroso-N-ethylaniline, formed gradually during storage, is estimated by means of the violet colour produced when it reacts with α-naphthylamine in presence of hydrochloric acid. Becker and Hunhold (*Z. Schiess. Sprengstoffw.*, 1938, **38**, 214) have since applied a modification of the method to the study of nitrocellulose powder stabilised with diphenylamine. The colorimetric reaction between aromatic N-nitrosoamines and α-naphthylamine hydrochloride has been shown by Parker (*J. Chem. Soc.*, 1946, 772) to be due to formation of the hydrochloride of 4-amino-1 : 1-azonaphthalene irrespective of the nitrosoamine employed. The method now presented is based on this reaction and serves to determine the total nitrosoamine content of cordite stabilised with *sym*-diethyldiphenylurea, and is applicable to the estimation of the thermal age of cordite and to the testing of materials for compatibility with cordite.

Procedure—Reduce the cordite to pass a B.S. Mesh Test Sieve No. 18. With artificially aged powder, allow the ground sample to stand for 24 hr.

at room temperature and then spread it on paper to aerate for 30 min. before weighing out the portion for the test.

Shake a small weighed portion of the prepared sample mechanically for 20 min. with purified 93 per cent. alcohol and adjust the mixture to a known volume. Allow the cordite to settle and take an aliquot containing up to the equivalent of 0·5 mg. of N-nitroso-N-ethylaniline. Owing to the photo-sensitivity of nitrosoamines the extraction and subsequent operations up to this stage should be carried out with the minimum exposure to light. After addition of 5 ml. of alcoholic 2 *N* hydrochloric acid and 5 ml. of 1 per cent. alcoholic α-naphthylamine solution, dilute the liquid to 90 ml. with 95 per cent. alcohol. Heat the liquid in a water-bath for 30 min. at 60° ± 2° C. and then place it in a thermostat at 20° C. for an hour. Finally, dilute the solution to 100 ml. with 95 per cent. alcohol and mix.

Determine the nitrosoamine concentration, calculated as N-nitroso-N-ethylaniline, by means of a Spekker absorptiometer (using a 1-cm. cell, a water-to-water setting of 1·00 and Ilford yellow-green filters, No. 605) by reference to a calibration curve obtained from known weights of N-nitroso-N-ethylaniline.

The method is rapid and convenient and determines 97 to 98 per cent. of the nitrosoamine present. Complete extraction can be made with hot ether in 2 hr. with a continuous extraction apparatus screened from light. Remove the ether carefully after extraction, blowing off the last few ml. at 20° C. Dissolve the residue in alcohol and proceed as already described. This modification is more suitable for compatibility testing, otherwise the more convenient extraction with alcohol is adequate.

Aged cordite SC contains brownish breakdown products partly soluble in alcohol. Interference from these is minimised by the filters used and is, in fact, negligible for samples having a thermal age not greater than 7 years at 49° C.

During the normal service life of cordite SC only two nitrosoamines are formed in noticeable amounts, *viz.*, N-nitroso-N-ethylaniline and its 4-nitro-derivative. After long periods of accelerated ageing very small amounts of the 2-nitro- and the 2 : 4-nitro-derivative are formed, and the full colour of these would not be developed in half an hour. The values obtained for "total nitrosoamine" content would then be slightly low, but not low enough to detract from the usefulness of the determination.

Compared with other stability tests the method described has the advantages that it is not empirical, it is unaffected by the presence of the distinguishing dyes sometimes added to cordite, it estimates directly a significant group of decomposition products and, with suitable modification, it can be applied to amounts of propellant far smaller than those used in other available methods.

A. O. JONES

Inorganic

New pH Indicator for Titration of Sodium Carbonate. Disodium 4 : 4′-bis-(2-amino-1-naphthylazo)-2 : 2′-stilbenedisulphonate. M. Taras (*Anal. Chem.*, 1948, **20**, 680–681).—The dyestuff, known as Hessian Purple N extra and Direct Purple, is prepared by tetra-azotisation of 9·5 g. of 4 : 4′-diaminostilbene-2 : 2′-disulphonic acid (Eastman Kodak T4614), and coupling with 8·0 g. of β-naphthylamine, which is first dissolved in 50 ml. of glacial acetic acid and diluted to 100 ml. The solution of the disodium salt is formed by grinding a weighed amount of the dyestuff with the calculated volume of 0·05 *N* sodium hydroxide and diluting to the required concentration. Its sharpest colour change is at pH 3·8, so that it is suitable for 0·2 and 0·5 *N* sodium carbonate. The alkaline solution is red, changing to faint mauve at 4·0, sharply to purple at 3·8, and to bluish-purple near 3·0. No colour standard is necessary, and artificial light is adequate.

Using a final concentration of 1 drop of 0·1 per cent. indicator solution for 10 ml. of solution, results on six amounts of sodium carbonate ranging from 0·1 to 1 g. were correct to within ±0·2 per cent. Comparable results are obtained by titrating samples of solid sodium carbonate.

M. E. DALZIEL

Determination of Hypochlorite in Presence of Chlorite. Determination of Hydrazine Sulphate. H. Caron and D. Raquet (*Chimie Analyt.*, 1948, **30**, 163–164).—The iodometric method of Poncius (Treadwell and Hall, *Analytical Chemistry*, Vol. 2, 7th Ed., p. 598) for the determination of hypochlorite is subject to error when chlorite is present. The method now described depends upon the following reaction in presence of sodium bicarbonate

$$2NaClO + NH_2NH_2.H_2SO_4 \rightarrow 2NaCl + H_2SO_4 + N_2 + 2H_2O$$

It is not affected by chlorite, which may subsequently be titrated iodimetrically in the same solution after acidification.

Procedure—Prepare a standardised solution of hydrazine sulphate containing 14·5 g. per litre. To 20 ml. of this solution add 1 ml. of a saturated solution of sodium bicarbonate and 1 g. of potassium bromide, then add the hypochlorite solution from a burette until a faint yellow colour indicates the liberation of a trace of bromine. If V = ml. of hypochlorite solution required, $100/V$ = strength of the solution in ml. of active chlorine per ml. If the strength is less than 5 or more than 10 ml. of active chlorine per ml., a correspondingly modified volume of hydrazine sulphate solution is used.

Determination of chlorite—Dilute the solution resulting from the hypochlorite determination to a suitable volume, take an aliquot, add a small amount of potassium iodide, acidify with dilute sulphuric acid, and titrate the liberated iodine with 0·1 *N* sodium thiosulphate. One litre of 0·1 *N* sodium thiosulphate is equivalent to 2·2625 g. of anhydrous sodium chlorite.

Determination of hydrazine sulphate—A solution of sodium hypochlorite of known strength is required and the procedure is the same as that used for the determination of hypochlorite.

W. C. Johnson

Polarographic and Amperometric Determination of Barium. I. M. Kolthoff and H. P. Gregor (*Anal. Chem.*, 1948, **20**, 541–544)—Barium ions give rise to polarographic reduction waves with a half-wave potential of $-1{\cdot}95$ v. *versus* the saturated calomel electrode, when 0·05 *M* calcium chloride is used as the supporting electrolyte. For solutions that are less than $1{\cdot}0 \times 10^{-3}$ *M* with respect to barium ions, the wave-height is proportional to barium concentration. When 0·1 *M* lithium chloride or 0·05 *M* magnesium chloride is used as supporting electrolyte, the reduction waves obtained are not suitable for analytical purposes.

The amperometric titration of barium with chromate ions can be carried out at an applied potential of $-1{\cdot}4$ v., using solutions containing from 20 to 50 per cent. of ethyl alcohol. The titration of solutions that are about 0·001 *M* with respect to barium gives results that are about 5 per cent. low, and if the barium concentration is 0·0001 *M*, the results are 10 per cent. low.

J. G. Waller

Precipitation of Oxalates from Homogeneous Solution. Application to Separation and Volumetric Determination of Magnesium. L. Gordon and E. R. Caley (*Anal. Chem.*, 1948, **20**, 560–563)—As usually obtained, the precipitate is difficult to filter and wash, but special devices to ensure slow precipitation give better products. Precipitation of magnesium oxalate in 85 per cent. acetic acid solution by slow hydrolysis of ethyl oxalate requires higher temperatures to obtain the best separation from lithium and sodium, methyl oxalate, as used for thorium, being too easily decomposed under these conditions. The precipitate so obtained is dense and coarsely crystalline, and does not adhere to the walls of the vessel. It can be filtered on sintered-glass or porcelain of medium porosity, and the nature of the precipitate enables more than 100 mg. of magnesium to be dealt with. A buffer, ammonium acetate, is necessary to control the decrease in pH due to the hydrolysis.

Although the magnesium to oxalate ratio is good in the dehydrated precipitate, high values, probably due to occlusion, are obtained by weighing the precipitates. Ignition to the oxide is satisfactory, but is troublesome and long.

Method—Adjust the volume of the neutral magnesium solution to 14 or 15 ml., add 75 ml. of glacial acetic acid and stir until dissolution is complete; then add 10 ml. of glacial acetic acid containing 1 g. of ammonium acetate. Add 1·5 ml. of ethyl oxalate and stir thoroughly. Place the covered beaker on a hot-plate to raise the temperature of the solution to 100° C. and maintain that temperature for 2 hr. after precipitation begins for up to 50 mg. of magnesium, and for 3 hr. for 100 mg. As a precautionary measure add, 15 min. before filtration, 5 ml. of 85 per cent. acetic acid solution saturated with ammonium oxalate at room temperature. Filter while hot, and transfer the precipitate to the filter crucible by means of 85 per cent. acetic acid solution at 70° to 80° C. and a rubber-tipped stirring rod. Wash then with 4 to 5 additional portions of about 5 ml. each. Rinse the suction flask thoroughly with distilled water, replace it, and dissolve the precipitate and wash the crucible with a total of 200 ml. of 5 per cent. sulphuric acid solution at 80° C. in small portions. Titrate the solution at once with potassium permanganate solution standardised against sodium oxalate at 70° to 80° C.

The permanganate normality should be adjusted to give a convenient titre.

Interfering substances are essentially those listed by Elving and Clay (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 558), but sulphate interferes more in this procedure and oxalate must be absent. In general application, after precipitating calcium as the oxalate, ammonium salts and excess oxalate are removed by treatment with nitric and perchloric acids, the excess of perchloric acid then being eliminated by fuming. The ethyl oxalate must be stored out of contact with moisture, as the partly hydrolysed material decomposes too rapidly.

Between 1 and 101 mg. of pure magnesium can be determined to within 0·3 mg. In the presence of from 10 to 100 mg. of lithium results are up to 0·5 mg. high on a similar range. Sodium up to 100 mg. does not interfere. A similar procedure may be adopted for precipitating the oxalates of zinc, cadmium, and lead.

M. E. Dalziel

Rapid Determination of Small Amounts of Silicon in Magnesium Alloys. D. F. Phillips and S. E. Hermon (*Metallurgia*, 1948, **38**, 179–180)—The method developed for the rapid determination of 0·02 to 0·25 per cent. of silicon in magnesium alloys containing up to 0·75 per cent. of zinc, 0·30 per cent. of manganese, 0·50 per cent. of copper, and 2 to 14 per cent. of aluminium is based on flocculation of the silica with gelatin. It gives good agreement with the usual sulphuric acid and perchloric acid methods. Twelve determinations can be made in about 2·5 hr. Highly oxidising conditions must be maintained during dissolution of the alloy or low results will be obtained.

Method—*Reagent*—*Brominated nitric acid*: 1 volume of saturated bromine water and 2 volumes of nitric acid (d. 1·42), to be made up freshly and well cooled. *Sulphuric acid mixture*: 6 volumes of diluted sulphuric acid (1 : 1) and 1 volume of 20-volume hydrogen peroxide. *Gelatin solution*: 0·25 per cent. prepared with hot, but not boiling, water and sterilised with a pea-sized crystal of thymol in a litre of solution. *Wash solution*: 10 ml. of diluted hydrochloric acid (1 + 1) and 20 ml. of 0·25 per cent. gelatin diluted to 1 litre.

Procedure—Add 50 ml. of brominated nitric acid to a 2-g. sample in a 500-ml. conical beaker. After dissolution of the sample, wash down the sides of the beaker with water and add 35 ml. of the sulphuric acid mixture. Cool, wash round with

10 to 20 ml. of water and add 40 ml. of gelatin solution with 2 filter accelerators. Stir vigorously, set aside for 45 min., filter through a Whatman No. 540 paper containing an accelerator, and wash 10 times with the wash solution. Dry the precipitate and ignite finally at 1000° C. C. F. HERBERT

Determination of Phosphorus in Hexa-ethyl Tetraphosphate and Tetra-ethyl Pyrophosphate. M. Jacobson and S. A. Hall (*Anal. Chem.*, 1948, **20**, 736–737)—Alkali-nitrate fusion converts the phosphorus into orthophosphate, and a colorimetric method of determination is preferred to a gravimetric method to avoid repeated ignitions. The yellow molybdivanadophosphoric acid method of Misson (*Chem. Ztg.*, 1908, **32**, 633), as modified by Kitson and Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 379), is used as nitrate is present.

Procedure—Standard graph—Dissolve 43·9 mg. of twice-recrystallised potassium dihydrogen phosphate in water in a 100-ml. volumetric flask, dilute to volume to obtain a solution containing 0·1 mg. of phosphorus per millilitre. Measure into 50-ml. volumetric flasks volumes ranging from 1 to 10 ml. (to within 0·01 ml.) of the solution and add to each 5 ml. of diluted nitric acid (1 + 2), 5 ml. of 0·25 per cent. ammonium vanadate solution containing 20 ml. of concentrated nitric acid per litre, and 5 ml. of 5 per cent. ammonium molybdate solution, and dilute the solutions accurately to 50 ml. Shake thoroughly and allow to stand for at least 10 min. Pour a portion into a clean, dry photometer test tube and measure its colour in a photo-electric photometer with a No. 46 blue filter, comparing with a blank solution of the nitric acid, ammonium vanadate, and ammonium molybdate solutions as 100 per cent. transmittance. Plot a curve on semi-logarithmic paper as percentage transmittance against concentration of phosphorus, and use it for reference for all analyses made with a given set of apparatus and reagents.

Fusion of sample—Weigh 40 to 50 mg. of sample into a small 7-ml. platinum crucible by means of a weighing pipette consisting of a glass tube with a fine capillary tip and a rubber bulb, the whole being supported in a wire holder. Add about 0·9 g. of an intimate mixture (4 : 1) of powdered sodium hydroxide and potassium nitrate, cover the crucible, and heat it gently until foaming ceases and then more strongly until a clear melt is obtained; about 5 to 6 min. are required. Allow to cool, and then dissolve the fused mass from the crucible in water and cover it with 75 ml. of hot distilled water. Transfer it to a 125-ml. Erlenmeyer flask. Acidify with concentrated nitric acid to Congo red and allow to simmer gently to reduce the volume to 50 ml. Cool the solution and nearly neutralise to litmus by means of aqueous ammonia solution (28 to 29 per cent.), transfer it to a flask, and dilute accurately to 100 ml. Treat a 5-ml. portion with 5 ml. each of diluted nitric acid (1 + 2), ammonium vanadate solution, and ammonium molybdate solution and dilute accurately to 50 ml. Measure the percentage transmittance and refer to the standard graph.

Attack on the platinum crucible is only slight, representing a loss of a few milligrams in 35 fusions, but the use of a gold crucible may be preferable.

M. E. DALZIEL

Polarographic Determination of Small Amounts of Arsenic [from Measurements of the Height of the "Maximum"]. N. Ya. Khlopin, N. A. Rafalovich, and G. P. Aksenova (*J. Anal. Chem. Russ.*, 1948, **3**, 16–20)—Under certain conditions the height of the maximum of arsenic obtained at applied potentials of −1·2 to −1·5 v. is linearly related to the arsenic concentration. Besides arsenic, essential components of the solution are: a weak acid, cations of cobalt, iron, or nickel, and an indifferent electrolyte.

The effects of various weak acids were studied in concentrations from 1 to 50 mg.-mol. per litre. In the presence of the other essential components, anthranilic and boric acids failed to give a maximum but formic, acetic, oxalic, malonic, succinic, lactic, tartaric, citric, benzoic, salicylic, monochloroacetic, and orthophosphoric acids were all effective.

Of cobalt, iron, and nickel cations, within the limits of 0·05 to 0·5 mg.-mol. per litre, the most effective was cobalt, but iron was found most convenient for ordinary use.

Variations of drop-time within the range 2 to 6·5 sec., and of the geometrical characteristics of the capillary had no appreciable effect on the form of the maximum.

Media suitable for the determination of 0·01 to 0·05 mg.-mol. of arsenious anhydride per litre are (1) 0·5 *M* in sodium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate, (2) 0·4 *M* in sodium sulphate, 0·35 *M* in sodium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate, and (3) 0·4 *M* in magnesium sulphate, 0·35 *M* in magnesium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate. Medium (1) allows a polarographic determination to be made directly on an arsenic distillate after neutralisation with sodium carbonate and addition of sodium chloride, (2) requires neutralisation of the distillate with sodium carbonate, and addition of sodium sulphate, and (3) requires neutralisation of the distillate with magnesium carbonate, and addition of magnesium sulphate.

Tests carried out with 1 to 5 µg. of arsenious oxide per ml. of a solution containing 0·2 g.-mol. of magnesium sulphate, 0·15 mg.-mol. of cobalt sulphate, and 15 mg.-mol. of a weak acid, showed that the height of the maximum (y) and the arsenic concentration (x) could be related by the formula, $y = bx + a$, where the coefficients b and a varied with the acid used, *e.g.*, under similar conditions, they were 2·6 and 5 for acetic acid, 2·6 and 10·2 for orthophosphoric acid, and 3·7 and 0 for succinic acid.

Samples of flour, paper, and bread were treated with known amounts of arsenic and the distillates obtained by the usual method were examined polarographically by the maximum method. The amounts found agreed to within about 5 parts per 100 with the amounts added. [Details of the procedure are not given.] G. S. SMITH

Reduction of Antimony Solutions with Metallic Nickel. H. Holness (*J. Soc. Chem. Ind.*, 1948, **67**, 238–241)—In order to apply the method to the removal of antimony in the tannin method for estimating tin in antimony-rich alloys, experiments on the influence of time, temperature, acid concentration, antimony concentration, and weight or area of nickel on the reduction of antimony trichloride in pure hydrochloric acid solution have been carried out. The solution must be able to make contact with the metal-hydrogen interface in the presence of both metals for reduction to proceed. The antimony deposit must thus be kept loose and porous and all the hydrogen must not be detached from the metal-solution interface.

Method 1, *powdered nickel*—Dissolve up to 1·8 g. antimony trioxide in 100 ml. of diluted hydrochloric acid (1 + 3). Heat on a water-bath. Add 5 g. of nickel powder and continue heating 20 min. without stirring or ebullition.

Method 2, *sheet nickel*—Prepare a spiral giving 30 sq. in. of surface. Clean by immersion in diluted nitric acid (1 + 3) until gassing freely and wash thoroughly. Activate by immersion in a boiling solution of 10 g. of sodium chloride, 25 ml. of hydrochloric acid, and 0·1 g. of antimony trioxide in 75 ml. water; remove after 2 to 3 min. when freely gassing and coated with a loose black deposit of antimony. Proceed as in Method 1.

C. F. Herbert

Colorimetric Determination of Copper with Carbon Disulphide and Diethanolamine. W. C. Woelfel (*Anal. Chem.*, 1948, **20**, 722–724)—A reagent prepared from carbon disulphide and diethanolamine reacts with cupric ions to form a water-soluble, brownish-yellow salt of *bis*-(2-hydroxyethyl) dithiocarbamic acid. Serious interference is caused by bismuth, chromium, cobalt, iron, mercury, nickel, silver, uranium, cyanide, dichromate, nitrite, and sulphite, but procedures are described for eliminating the interference of appreciable amounts of bismuth, chromium, iron, and uranium. It is suggested that the new reagent be named *cuprethol*.

Reagents—(*a*) *Acetate buffer solution pH* 5—Dissolve 96 g. of sodium acetate trihydrate and 17 ml. of glacial acetic acid in water and dilute to 1000 ml. (*b*) *Cuprethol reagent—Solution A*—Dissolve 4 g. of diethanolamine in 200 ml. of methyl alcohol. *Solution B*—Dissolve 1 ml. of carbon disulphide in 200 ml. of methyl alcohol. Prepare sufficient reagent for one day's use by mixing equal volumes of Solutions A and B. (*c*) *Standard copper solution*—Dissolve 0·1000 g. of pure copper in 5 ml. of 8 *N* nitric acid, add 1 ml. of concentrated sulphuric acid, and evaporate to dryness. Dissolve the residue in water and dilute to 1000 ml.; 1 ml. contains 0·1 mg. of copper. Dilute 50 ml. of this solution to 1000 ml.; 1 ml. of this solution contains 0·005 mg. of copper.

Procedure for samples of low iron content—The solution to be tested should contain between 2 and 10 mg.-equivalents of mineral acid and not more than 10 mg. of iron and 0·2 mg. of copper. Dilute to approximately 50 ml. with water. Add 1 ml. of a 3·0 per cent. solution of sodium pyrophosphate ($Na_4P_2O_7.10H_2O$) for each 2 mg. of iron present and adjust the reaction of the solution to a pH of 5 to 6 by adding 20 per cent. sodium acetate solution. If no turbidity develops in 5 min., add 1 ml. of cuprethol reagent, dilute to 100 ml. with water, and within 1 hr., measure the colour intensity in a photometer.

Procedure for samples of high iron content—If the sample contains large amounts of iron or other metals precipitated by pyrophosphate, remove them by careful precipitation with 15 *N* ammonia solution. Dilute an aliquot of the ammoniacal filtrate containing not more than 0·2 mg. of copper to approximately 50 ml. with water, and make just acid to litmus with 1·2 *N* hydrochloric or nitric acid. Add 10 ml. of acetate buffer solution, 1 ml. of cuprethol reagent, dilute to 100 ml. with water, and within 1 hr. measure the colour intensity in a photometer.

Determine the copper content of the aliquot by reference to a standard curve prepared from the standard copper solutions. Beer's law is valid for concentrations up to 0·2 mg. of copper per 100 ml. of solution.

Interfering ions—Drabkin (*J. Assoc. Offic. Agr. Chem.*, 1939, **22**, 320) has shown that potassium cyanide destroys the colour formed by cupric ions with diethyldithiocarbamate in that process for the estimation of copper. The principle has been utilised to nullify the interference of up to 10 p.p.m. of bismuth, 300 p.p.m. of uranium, and 100 p.p.m. of chromium by making photometer readings of the coloured solution before and after addition of a small amount of potassium cyanide: the copper is determined by difference.

The presence of tungsten causes a green colour to develop early, but this disappears after a few minutes and does not interfere in the copper determination.

With more than 200 p.p.m. of thiosulphate, interference is caused by the precipitated sulphur. Dichromate oxidises the cuprethol reagent and gives gross negative errors in concentrations above 2 p.p.m. Twenty p.p.m. of nitrite or 100 p.p.m. of sulphite or oxalate can be tolerated if the photometer readings are obtained within 10 min. Not more than 20 p.p.m. of cyanide may be present.

The presence of mercuric ions inhibits colour development.

A. H. A. Abbott

Ammonium Citrate in the Colorimetric Determination of Copper. A. J. Hall and R. S. Young (*Anal. Chem.*, 1948, **20**, 776–777)—Because large amounts of mercury interfere with the determination of copper with sodium diethyldithiocarbamate, it is proposed to measure the blue colour of a copper ammonium citrate complex directly in presence of mercury, which is held in solution by ammonium citrate and an excess of ammonia. Under these conditions variations in the quantity of mercury present have no effect on the colour due to the copper, and an adequate amount of mercuric chloride is provided in the solutions used to prepare a standard curve.

Reagent—Dissolve 200 g. of citric acid in water, add 270 ml. of aqueous ammonia (S.G. 0·88) and dilute to 1000 ml.

Procedure to obtain standard curve—(*a*) 0 *to* 50 *mg. of copper*—From a solution of copper sulphate containing 1 mg. of copper per ml., measure volumes containing up to 50 mg. of copper in 5- or 10-mg. increments, and to each add 50 ml. of 7 per cent. mercuric chloride solution, 50 ml. of ammonium citrate solution, and 50 ml. of aqueous ammonia (sp.gr. 0·88). Dilute to 200 ml. with water and measure the absorption of the solution in a 4-cm. cell. (*b*) 0 *to* 200 *mg. of copper*—From a solution of copper sulphate containing 5 mg. of copper per ml., measure volumes representing up to 200 mg. of copper in 10-mg. increments, and to each add mercuric chloride solution, ammonium citrate solution, and aqueous ammonia, as above. Dilute to 200 ml. with water and measure the absorption in a 1-cm. cell.

To determine the copper content of unknown solutions containing mercury, add ammonium citrate solution and ammonia, dilute to 200 ml. with water, and compare the absorptiometer reading with the standard curves.

Copper may be similarly determined in presence of silver, zinc, cadmium, magnesium, aluminium, and lead. Solutions containing coloured ions, *e.g.*, cobalt, chromium, and nickel, cause interference and more than 10 mg. of iron imparts a green tinge. The presence of chloride, acetate, and nitrate ions has no effect on the copper colour.

A. H. A. ABBOTT

1 : 2-*Cyclo*hexanedione Dioxime (Nioxime). Reagent for Nickel. R. C. Voter, C. V. Banks, and H. Diehl (*Anal. Chem.*, 1948, **20**, 458–460; *cf.* Johnson and Simmons, *Analyst*, 1946, **71**, 554)—The sensitivity of the reagent for nickel is 1 in 10^7, and quantitative precipitation occurs at pH values of 3·0 and greater. In gravimetric work, the precipitate is obtained in a readily filterable form by slowly raising the pH of the solution from a point where the nickel complex does not precipitate to a value of about 4·5. The precipitate contains co-precipitated reagent but the error arising from this cause under the standardised conditions, is a linear function of the excess of nioxime and is corrected by the application of a factor. The correction is small enough to be neglected when the quantity of nickel is less than 15 mg.

Procedure—To the solution containing about 25 mg. of nickel in 250 ml. add 8 ml. of a 0·8 per cent. aqueous solution of nioxime for each 10 mg. of nickel present. Add sufficient hydrochloric acid to re-dissolve any red precipitate formed and then ammonia until a faint red colour persists. Heat to 60° C. and add, dropwise with constant stirring, 25 ml. of 20 per cent. ammonium acetate solution. Maintain at 60° C. for 30 to 40 min. with occasional stirring, filter through a crucible of medium porosity, wash with hot water, and weigh. The volume of solution actually used to precipitate the nickel = weight of precipitate × 104. Correction in g. of nickel = 0·0002 × excess of nioxime per cent. × weight of precipitate × 0·1721.

Four determinations of quantities of nickel 1·8 to 14·4 mg. show in each an error of 0·1 mg. and a determination of 20·3 mg. shows no error.

The following ions present in solution do not interfere with the nickel determination: acetate, Cl', SO_4'', NO_3', ClO_4', tartrate, sulphosalicylate, $UO_2^{\cdot\cdot}$, $Mn^{\cdot\cdot}$, $Na^{\cdot}$, $K^{\cdot}$, $Li^{\cdot}$, $Ba^{\cdot\cdot}$, $Ca^{\cdot\cdot}$, $Sr^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $Cd^{\cdot\cdot}$, antimonite (+ tartrate), aluminium (+ tartrate), AsO_2', $Be^{\cdot\cdot}$, and $Zn^{\cdot\cdot}$. Copper gives a brownish-green colour and cobalt is co-precipitated. No satisfactory method can be recommended for the quantitative separation of nickel from iron by nioxime. Ferrous iron forms a stable complex with nioxime. Nioxime will also reduce ferric to ferrous iron.

W. C. JOHNSON

Photo-colorimetric Determination of the $Co^{\cdot\cdot}$ Ion by means of α-Nitroso-β-naphthol and β-Nitroso-α-naphthol. A. L. J. Beguet (*Anal. Asoc. Quim. Argentina*, 1946, **34**, 105–126)—Compounds containing the grouping –CO–C=N–OH have been proposed (Illinski and von Knorre, *Ber.*, 1885, **18**, 699) for the determination of small amounts of cobalt. The reagents most frequently used are the following: α-nitroso-β-naphthol (Bellucci, *Gazz. Chim. Ital.*, 1919, **49**, II, 294), β-nitroso-α-naphthol (Baudish *et al.*, *Ber.*, 1912, **45**, 1164; 1915, **48**, 1610), *o*-nitrosophenol (Feigl, *Specific and Special Reactions*, p. 109, Elsevier Publishing Co., Inc., New York, 1941), nitrosoresorcinol (Tonucek and Komarek, *Z. anal. Chem.*, 1932, **91**, 29B; Novelli, *Actas y Trabajos del II Congreso de Quimica* (I Sudamericano), Buenos Aires, 1924; *Idem.*, *Ibid.*, Vol. II, p. 117), 2-nitroso-1-naphthol-4-sulphonic acid (Sarver, *Ind. Eng. Chem.*, *Anal. Ed.*, 1938, **10**, 378), nitroso-R-salt (*Anal. Asoc. Quim. Argentina*, **31**, 124), and *iso*nitroso-acetophenone (Feigl, *Ibid.*).

The present author has determined the limits of the application of Beer's law in the photo-colorimetric determination of the $Co^{\cdot\cdot}$ ion with α-nitroso-β-naphthol and with β-nitroso-α-naphthol, both in the aqueous phase and in ethyl acetate, using Pulfrich and Hellige photometers. The interfering effect of the ions $Ni^{\cdot\cdot}$, $Cu^{\cdot\cdot}$, and $Cr^{\cdot\cdot\cdot}$ in the aqueous solution, and of $Ni^{\cdot\cdot}$ and $Cr^{\cdot\cdot\cdot}$ in ethyl acetate have been investigated.

Reagents—3 *M* aqueous ammonia; ammonium citrate solution, 0·6 g. per ml.; 0·1 per cent. solution of α-nitroso-β-naphthol (prepared according to Snell and Snell, *Colorimetric Methods of Analysis*, Vol. I, pp. 324 *et seq.*, D. van Nostrand, New York, 1936); 0·1 per cent. solution of β-nitroso-α-naphthol (*Idem.*, *Ibid.*); 0·1 *M* solution of cobalt, prepared from $CoCl_2.6H_2O$ and standardised by the electrolytic method.

Determination of the $Co^{\cdot\cdot}$ ion by means of α-nitroso-β-naphthol. Aqueous solution—The most suitable colour filter was determined by measuring the light transmission at different wavelengths through a solution of α-nitroso-β-naphthol and through one of the cobalt complex. Curves from which could be read the wavelength that gave the maximum sensitivity and the minimum interference by the reagent were then constructed. This wavelength was 530 mμ.

In the determination, the order of addition of the reagents is important. To prepare a solution of the complex that contains x p.p.m. of $Co^{\cdot\cdot}$, add to x ml. of a solution containing 10 p.p.m. of cobalt, 0·5 ml. of ammonium citrate solution and then 0·5 ml. of 3 M aqueous ammonia, dilute to 9 ml. with water, and then add 1 ml. of α-nitroso-β-naphthol solution, and shake. Beer's law is obeyed from 0 to 2 p.p.m. The lower limit of determination of the cobalt ion by this method is 0·025 p.p.m. The results obtained with a Pulfrich photometer differ very slightly from those of Burkhardt (Doctoral Thesis, F.C.E.F. y N., Buenos Aires, 1943), who used an Aminco photo-electric photometer, and who found Beer's law to be obeyed up to 0·05 p.p.m. With the Hellige photometer, the limits of conformity to Beer's law were 0 to 2·4 p.p.m. and the lower limit of cobalt that could be determined was 0·5 p.p.m.

Determination of the $Co^{\cdot\cdot}$ ion by means of β-nitroso-α-naphthol. Aqueous solution—The determination is carried out as before, the optimum wavelength being 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m. of cobalt ion, and the lower limit of determination of the ion is 0·025 p.p.m.

Determination of the $Co^{\cdot\cdot}$ ion by means of α-nitroso-β-naphthol. Ethyl acetate solution—The reaction is carried out in a separating funnel, into which are put 1·25 ml. of ammonium citrate solution, x ml. of cobalt salt solution of z p.p.m., and 1·25 ml. of 3 M aqueous ammonia solution; the volume is brought to about 20 ml. with water, and, after shaking, 2·5 ml. of the alkaline 1 per cent. α-nitroso-β-naphthol solution are added, the volume is made up to 25 ml. with water, and the funnel is shaken. There are then added 7·5 ml. of ethyl acetate, and the funnel is shaken vigorously and set aside for the phases to separate. The aqueous phase is run off, the tap being opened carefully and only partially; the tap is closed and the funnel rotated horizontally until the last drops of water have separated, whereupon they are run off. The ethyl acetate phase is run out through the neck of the funnel, the first two portions being used to wash out the photometer cells and the third used for the measurement; the compensation liquid is also ethyl acetate. It is important in all these determinations that the cells be washed out at least twice with the liquid on which measurements are to be made. With ethyl acetate, which is volatile, the cells must be completely full and the measurements must be made quickly. The readings should not be taken in bright light.

Curves were constructed as before, one in which wavelength is plotted against the light transmitted by a 0·01 per cent. solution of the reagent in ethyl acetate, and the other similarly for a solution of reagent and 0·3 p.p.m. of cobalt ion. The wavelength giving maximum sensitivity and minimum interference is 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m., and the smallest quantity of cobalt determinable is 0·01 p.p.m.

Determination of the $Co^{\cdot\cdot}$ ion with β-nitroso-α-naphthol. Ethyl acetate solution—The determination is carried out exactly as with α-nitroso-β-naphthol in ethyl acetate. The optimum wavelength is 530 mμ. Beer's law is obeyed from 0 to 0·3 p.p.m. of cobalt ion, and the smallest quantity of cobalt determinable is 0·01 p.p.m. With the Hellige photometer, Beer's law is obeyed from 0 to 0·14 p.p.m., and the smallest amount that can be determined is 0·02 p.p.m.

Determination of the $Co^{\cdot\cdot}$ ion in presence of the $Ni^{\cdot\cdot}$ ion, with α-nitroso-β-naphthol. Aqueous solution—The only additional reagent is a 0·06 M solution of nickel chloride, prepared from pure $NiCl_2.6H_2O$, and standardised electrolytically. The Hellige photometer is used. To determine the optimum wavelength for the measurements, curves are prepared, as before, relating transmission to wavelength for a 0·05-p.p.m. [0·5?] nickel solution, for the same nickel solution in the presence of a 0·01 per cent. solution of α-nitroso-β-naphthol, and for a solution containing nickel, α-nitroso-β-naphthol, and 1 p.p.m. of cobalt ion. [The optimum wavelength found is not stated.] The technique of the determination is the same as for the direct determination with α-nitroso-β-naphthol in aqueous solution, except that sufficient of the nickel solution to give a concentration of 0·5 p.p.m. is added. Beer's law is obeyed between 0 and 0·8 p.p.m., and the smallest quantity of cobalt determinable is 0·025 p.p.m., the same concentration as can be determined in absence of nickel.

Determination by means of β-nitroso-α-naphthol in presence of nickel. Aqueous solution—The optimum wavelength is 530 mμ. The Hellige photometer was used. Beer's law is obeyed between 0 and 1 p.p.m., in presence of 0·5 p.p.m. of nickel; the smallest quantity of cobalt that can be determined is 0·025 p.p.m.

Determination by means of α-nitroso-β-naphthol in the presence of 0·5 p.p.m. of $Cu^{\cdot\cdot}$ ion. Aqueous solution—The additional reagent required is a 0·024 M solution of copper sulphate, prepared from pure, recrystallised $CuSO_4.5H_2O$, and standardised by the electrolytic method in a medium containing sulphuric and nitric acids. Two curves were constructed, with the Hellige photometer, one for light transmission at different wavelengths by a 0·01 per cent. solution of the reagent plus 0·5 p.p.m. of copper, and the second for a solution containing 0·01 per cent. of the reagent, 0·5 p.p.m. of copper and 1 p.p.m. of cobalt. The optimum wavelength is 530 mμ. The technique is as before, with addition of sufficient of the copper solution to give a concentration of 0·5 p.p.m. Beer's law is obeyed between 0 and 0·8 p.p.m. of cobalt, and the smallest quantity of cobalt that can be determined is 0·05 p.p.m.

Determination by means of β-nitroso-α-naphthol in the presence of the $Cu^{\cdot\cdot}$ ion. Aqueous solution—Curves were constructed as in the previous example, with the Hellige photometer. The optimum wavelength is 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m. of cobalt ion, and the smallest concentration that can be determined is 0·05 p.p.m.

Determination by means of α-nitroso-β-naphthol in the presence of the $Cr^{\cdot\cdot\cdot}$ ion. Aqueous solution—Re-crystallised chrome alum was used to prepare a 0·25 M solution, which was standardised by the gravimetric method, as Cr_2O_3, calcined in a current of hydrogen. Three curves were prepared, with the Hellige photometer, the first relating the light transmission through a 25 p.p.m. chromium solution to the wavelength, the second for a 0·1 per cent. solution of α-nitroso-β-naphthol containing 25 p.p.m. of chromium, and the third for this same solution containing 1 p.p.m. of cobalt ion. The optimum wavelength was 530 mμ. Further increase in the chromium concentration causes a turbidity due to the formation of chromium hydroxide. Beer's law is obeyed between 0 and 1·4 p.p.m. of cobalt, and the smallest quantity determinable is 0·025 p.p.m.

Determination by means of β-nitroso-α-naphthol in the presence of the $Cr^{\cdot\cdot\cdot}$ *ion. Aqueous solution*—The Hellige photometer was used. Beer's law is obeyed between 0 and 1·2 p.p.m. of cobalt, and the smallest quantity of cobalt determinable is 0·05 p.p.m.

Determination by means of α-nitroso-β-naphthol in the presence of the $Ni^{\cdot\cdot}$ *ion. Ethyl acetate phase*—Curves are prepared as before, using a 0·01 per cent. solution of the reagent with 2 p.p.m. of nickel, and this solution with the addition of 2 p.p.m. of cobalt ion. The optimum wavelength is 530 mμ. The Hellige photometer was used. Beer's law is obeyed between 0 and 0·08 p.p.m. of cobalt, and the smallest quantity of cobalt determinable is 0·01 p.p.m.

Determination by means of β-nitroso-α-naphthol in the presence of 2 p.p.m. of nickel. Ethyl acetate phase—The optimum wavelength is not stated. The Hellige photometer was used. Beer's law is obeyed between 0 and 0·12 p.p.m. of cobalt, and the smallest quantity of cobalt that can be determined is 0·02 p.p.m.

Determination by means of α-nitroso-β-naphthol in the presence of 10 p.p.m. of the $Cr^{\cdot\cdot\cdot}$ *ion. Ethyl acetate phase*—Curves are prepared for solutions containing 0·01 per cent. of the reagent and 0·10 [10?] p.p.m. of chromium, and containing 0·01 per cent. of the reagent, 0·2 p.p.m. of cobalt and 10 p.p.m. of chromium. The Hellige photometer was used. The optimum wavelength was 530 mμ. Beer's law is obeyed between 0 and 0·2 p.p.m. of cobalt, and the smallest concentration of cobalt determinable was 0·02 p.p.m.

Determination by means of β-nitroso-α-naphthol in the presence of 10 p.p.m. of chromium. Ethyl acetate phase—Curves are prepared as before, both solutions containing 10 p.p.m. of chromium. The Hellige photometer was used. The optimum wavelength was 530 mμ. Beer's law is obeyed between 0 and 0·1 p.p.m. of cobalt, and the smallest concentration of cobalt that can be determined is 0·02 p.p.m.

E. M. Pope

1 : 2-*Cyclo*hexanedione Dioxime. A Reagent for Palladium. R. C. Voter, C. V. Banks, and H. Diehl (*Anal. Chem.*, 1948, 20, 652–654)—The reagent, which is water-soluble, yields with palladium an insoluble yellow compound that can be filtered from a hot solution after a brief period of digestion. Slight yellow colorations are obtained with 5, 7, or 10 parts per 10 million after 5 min., whilst dimethylglyoxime shows no reaction, although both reagents give slight precipitates on standing several hours.

Quantitative precipitation is obtained at pH values between 0·7 and 5 in presence of an excess of between 30 and 150 per cent. of the reagent. From 6 to 30 mg. of palladium can be determined to within 1 part in 135, but filtration of larger quantities is difficult.

Procedure—Adjust the volume of the solution containing 5 to 20 mg. of palladium to about 200 ml., and the pH to between 1 and 5 depending on other cations. Heat the solution to about 60° C. and add slowly from a pipette, with constant stirring, 0·43 ml. of a 0·8 per cent. solution of the reagent for each milligram of palladium present; this provides a 30 per cent. excess. Digest with occasional stirring for 30 min. at 60° C., filter through a medium-porosity crucible and wash the precipitate with 5 portions of hot water. Dry at 110° C. for 1 hr. The compound contains 27·43 per cent. of palladium.

In presence of platinum the same procedure applies, the platinum compound separating only after more than 24 hr. standing; spectrographic examination shows that separation is better than with dimethylglyoxime. Sulphate, nitrate, chloride, acetate, tartrate, and sulphosalicylate do not interfere, nor do uranyl, beryllium, ruthenium, aluminium, lanthanum, zinc, calcium, alkali metals, or alkaline earths, but aurous ions at 60° C. give a precipitate contaminated with metallic gold.

M. E. Dalziel

Mercaptobenzothiazole as a Reagent for Platinum, Rhodium, and Palladium. I. Ubaldini (*Gazz. Chim. Ital.*, 1948, 78, 293–301)—Mercaptobenzothiazole is a sensitive reagent for determining platinum, rhodium, and palladium, giving visible precipitates in solutions containing about 10 μg. of metal per 100 ml.

The reagent is prepared by dissolving the thiazole in alcohol, alkali, or acetic acid.

Platinum is precipitated quantitatively in acid solution. In alkaline solution no precipitate is formed but, on acidifying with acetic or hydrochloric acid and boiling, a canary yellow, flocculent precipitate that filters very readily is produced. It is washed with hot water, dried, and incinerated in a porcelain or quartz crucible and weighed as the metal. The precipitate is insoluble in boiling concentrated hydrochloric acid, or in 25 per cent. nitric acid.

Rhodium is completely precipitated in alkaline solution or in presence of acetic acid, but only imperfectly in presence of mineral acids. On boiling the alkaline solution the flocculent, red-brown precipitate forms immediately and filters very readily. The metal is obtained by incinerating in a porcelain crucible followed by ignition in a current of hydrogen.

Palladium behaves like platinum in acid solution, but can also be precipitated in presence of alkali.

A. H. Bennett

Physical Methods, Apparatus, etc.

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 683–684)—Data for two modifications of thiamine hydrochloride (vitamin B_1) are given (*cf. Ibid.*, 1948, **20**, 275; *Analyst*, 1948, 73, 579). M. E. DALZIEL

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 779–780)—Crystal properties of α-pyridinesulphonic acid in three polymorphic forms, only one being well-defined, are described under the headings given in the original publication (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

Storage and Titration with Oxygen-Sensitive Solutions. H. W. Stone (*Anal. Chem.*, 1948, **20**, 747–749)—The system described uses nitrogen under a slight pressure and avoids the necessity of maintaining inert gas pressure by means of a Kipp's apparatus or a cylinder, and also of scrubbing the inert gas over a long period.

Apparatus—In Fig. 1 the gas pressure compensating systems for storage and the burette are separate.

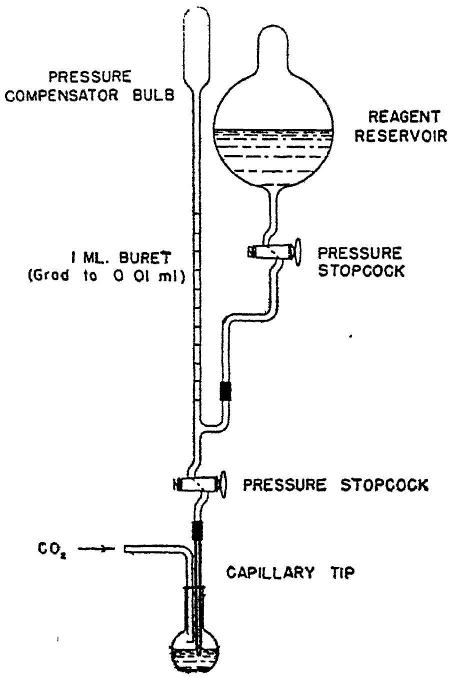


Fig. 1

The nitrogen pressure in the reservoir, made from a round-bottomed flask, must be just sufficiently greater than that in the burette to allow the burette to fill, and that in the burette must be high enough to expel the solution during titration. The apparatus shown in Fig. 2 requires a reservoir above the burette graduations, as gravity controls the flow of solution; thus, only one pressure adjustment is necessary in preparing the apparatus, but if the burette is allowed to empty, the nitrogen becomes contaminated and the whole system must be recharged.

Introduction of reagent—The necessary apparatus is shown in Fig. 3, the top stop-cock representing the burette tap in Fig. 2. At the start, screw-clamps B, C, and E are open and D is closed. Pass air at 2 atmospheres pressure and vent through B, which is gradually closed until the chromic chloride solution in hydrochloric acid is forced up through the reductor, through E to C, thus clearing air from the line. Open the burette stop-cock, close C, and regulate the flow into the reservoir (Fig. 2) by adjusting B. When enough solution is in the burette and reservoir to absorb all the oxygen, close the stop-cock and E and shake the apparatus, allowing nitrogen through D and the stop-cock to compensate for the decrease in pressure. The pressure of nitrogen should be reduced to about 1 atmosphere by means of a valve on the cylinder. Close the stop-cock and shake the apparatus again lest the nitrogen contains any oxygen. Clear the storage flask of the partly-spent solution and the excess of nitrogen by closing D and opening the burette stop-cock and C and adjusting the pressure to 1 atmosphere by venting through C. Then charge with the reagent by closing C, opening E and forcing the solution up with B closed until the reservoir is half full, the pressure then being 2 atmospheres.

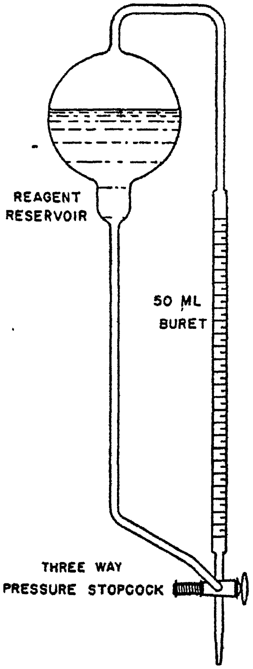


Fig. 2

To dispense oxygen-free water, pump out the system with an oil pump and fill with oxygen-free nitrogen alternately until the partial pressure of oxygen is adequately reduced, then admit distilled water, previously boiled and cooled in a stream of nitrogen.

Nitrogen can be replaced by hydrogen, but carbon dioxide is not suitable as its solubility is too variable. Diffusion of oxygen through the rubber is compensated for by replacing the reagent in the lines with fresh solution before a fresh group of titrations. During titrations a capillary tip is fitted to the burette and allowed to dip below the surface, so restricting diffusion and oxidation, while commercial carbon dioxide is blown over the liquid surface at a rate of 200 to 300 ml. per min. The gas stream is started about 5 min. before titration to sweep out the vessel, but longer time is necessary to clear the line from the cylinder.

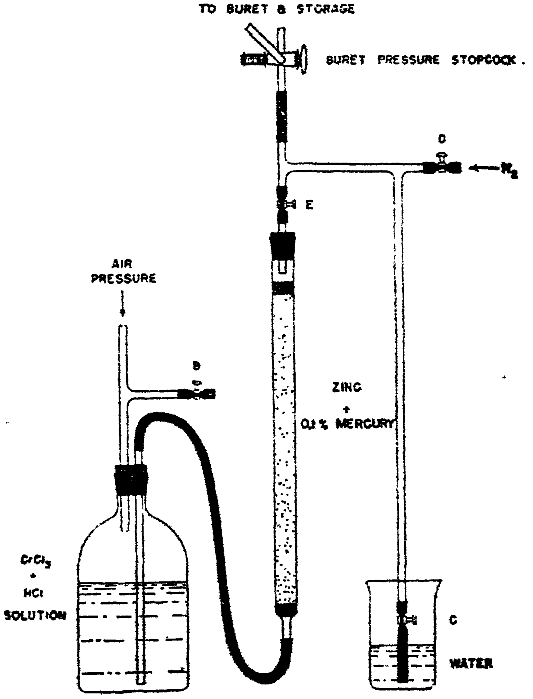


Fig.

Solutions of 0·03 N chromous chloride in hydrochloric acid showed no variation in titre in 10 months. The apparatus requires no attention when not in use, and is ready at a moment's notice.

M. E. Dalziel

Automatic Potentiometric Titrations. J. J. Lingane (*Anal. Chem.*, 1948, **20**, 285–292)—Potentiometric titrations applicable to any type of electrode reaction can be carried out with the apparatus described, which will either record the complete titration curve or stop the titration at the equivalence point. The titrant is delivered from a 50-ml. hypodermic syringe, the plunger being driven by a screw thread turned by a synchronous motor. The number of rotations of the screw are recorded by a revolution counter and the syringe is calibrated by weighing the water forced out by a definite number of complete rotations of the screw. Since the plunger moves at a constant speed, the volume delivered can also be related to the time of delivery.

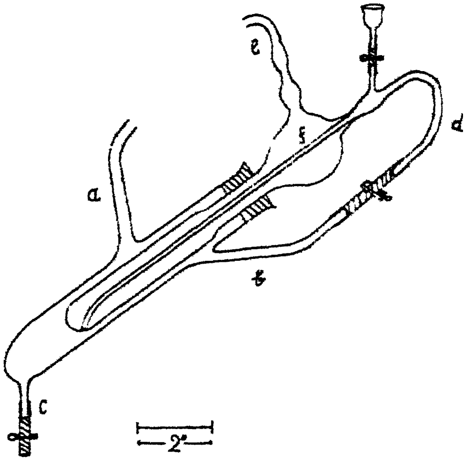
An indicator electrode and a saturated calomel reference electrode are coupled to a recording potentiometer fitted with a mercury switch, which can be set to stop the titration at the equivalence point, if this is desired. The nature of the indicator electrode depends on the electrode reaction taking place, and is usually a strip of an appropriate metal.

Since additions can be made more accurately from a syringe than from a burette of the same volume, this apparatus is capable of giving results that are more accurate than those obtained by the usual methods. By using a syringe of smaller volume, the method can be applied on the semimicro scale.

For routine purposes in which the shape of the titration curve is known, the recording potentiometer is replaced by any potentiometer control of rapid response, set to stop the titration at the equivalence point.

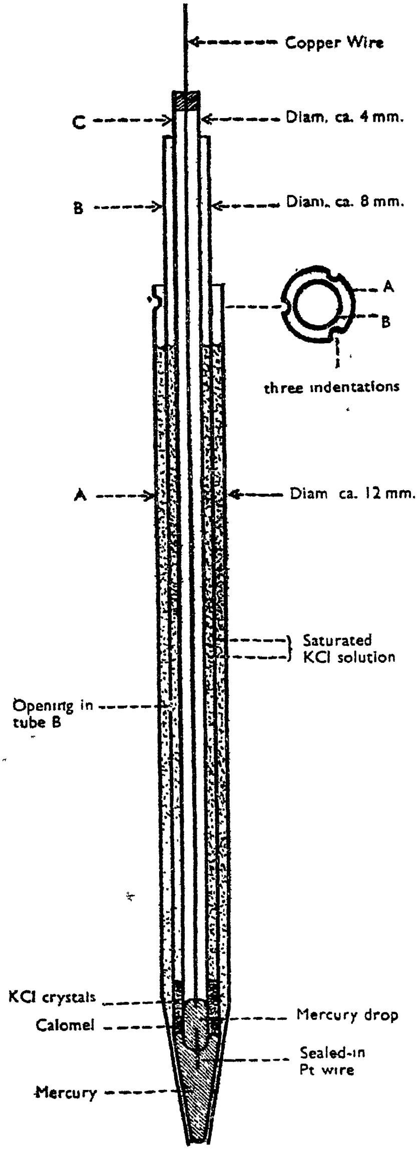
J. G. Waller

"Micro"-Kjeldahl Distillation Apparatus. R. Johanson (*J. Proc. Austral. Chem. Inst.*, 1948, **15**, 183–184)—In the apparatus shown the outer jacket serves as a steam jacket for the inner distillation tube and as a vacuum jacket for collecting the spent sample, the inner tube being suspended in it by means of a rubber stopper. The sample is admitted through the thistle funnel, and the clip on the connection closed; steam is then allowed to enter through the tube *a*, is led through *b* into *d* and tube *f*, and bubbles through the sample liquor into *e* and the condenser, which is not shown. Distillation is rapid and smooth, and emptying out of the spent liquor into the outer jacket is automatic at the close of a determination.



M. E. Dalziel

Calomel Electrode. B. van der Burg (*Chem. Weekblad*, 1948, **44**, 417–418)—The simple and easily made calomel electrode described is composed of a glass tube, A, of 140 mm. length, drawn out at the lower end and provided with three indentations at the upper end. A second tube, B, inserted into the first one, has the lower end drawn out and sealed off, the drawn-out portion being ground into the corresponding part of the tube A. In B is placed a small amount of re-distilled mercury, then some calomel and a layer of potassium chloride crystals, after which the innermost tube, C, which is closed at the bottom and has a platinum wire sealed through the lower end, is inserted into the tube B. Contact is made with the platinum wire by means of a bead of mercury in the tube C and an amalgamated copper wire. At the upper end this tube is closed by wax. Finally, the tubes A and B are filled with saturated potassium chloride solution. The prepared electrode is placed in the liquid of which the pH is to be determined. After use it is washed with distilled water and dried with filter paper, and a drop of potassium chloride solution is allowed to flow out. The level of the liquid in the electrode must always be above the hole connecting the two tubes. G. Middleton



Chemical Analysis Based on X-ray Absorption Measurements with a Multiplier Phototube. I. Solids and Liquids. H. A. Liebhafsky, H. M. Smith, H. E. Tanis, and E. H. Winslow. II. Gases. E. H. Winslow, H. M. Smith, H. E. Tanis, and H. A. Liebhafsky (*Anal. Chem.*, 1947, **19**, 861–865, 866–867)—The use of X-ray absorption as a method of chemical analysis has long been appreciated, but simple and precise measurement of X-ray intensity has only recently become possible. These papers describe how the method may be used when X-ray intensity is measured by means of a phosphor and multiplier photo-tube coupled to an amplifier.

Method—Polychromatic X-rays are passed through sheets of the material being analysed or, for gases, liquids, or divided solids, through a cell in which a known weight of sample is placed. The absorption is then measured and the mass absorption coefficient deduced. Absorption, being an atomic property, independent in general of the physical state, depends upon the elements and their relative proportions present in the sample. The method of analysis is based upon this principle.

A constant-voltage source is necessary for accurate work.

Results—**Solids and Liquids**—Preliminary experiments show that materials such as polystyrene, saflex, butvar, ethyl cellulose, cellulose acetate, cellophane, koroseal, and saran, can be distinguished from one another.

The uniformity and, very roughly, the quality of coal can be assessed in a few minutes, and the chlorine contents of chlorinated hydrocarbon polymers determined in about one-tenth the time required for the conventional analysis.

A limitation of the method is exemplified by methyl alcohol and sucrose which give identical absorption coefficients.

This ambiguity arises from the use of polychromatic X-rays, changes in "effective wavelength" of the X-rays occurring from one measurement to the other. The "effective wavelength" of a polychromatic beam is defined as that of a monochromatic beam absorbed to the same extent under the experimental conditions.

Gases.—Measurements made on hydrogen, methane, air, oxygen, methyl chloride, and chlorine, show that, as would be expected, the softest radiation is best for analytical work, but it is found that when longer wavelengths are used, particular attention must be paid to changes in the "effective wavelength."

The temperature and pressure of the gas should be known in order to calculate the density of the sample.

Curves for the absorption of methyl chloride at reduced pressures are given. E. G. Steward

Reviews

TRACE ELEMENTS IN FOOD. By G. W. MONIER-WILLIAMS, O.B.E., M.C., M.A., Ph.D., F.R.I.C. Pp. viii + 511. London: Chapman & Hall Ltd. 1949. Price 30s. net.

It is not so long since "trace elements" in food would have been defined as elements known or suspected to occur naturally and thought to exert vital functions in the life cycle, but present in such minute amounts as not to be quantitatively assessable. The emphasis would have been on the word trace to indicate a lack of precise knowledge. Within recent times, however, analytical chemistry, particularly in its spectrographic and colorimetric branches, has advanced and developed to such purpose that it is now possible to obtain at least close approximations for the smallest amounts of these elements. In the preface to his book Dr. Monier-Williams remarks that there is for most trace elements a wide range between those quantities that are by common consent innocuous or even essential to health and those that show signs of being injurious; and that to decide on the limits within this range to which foods should conform is not easy. To any food chemist this remark applies with particular force. The difficulty arises in assessing the validity of much that may be regarded as having been accepted by "common consent." There may be little doubt of a minimum toxic amount, yet a grave doubt of the minimum amount properly to be regarded as potentially harmful in the circumstances of differing chemical combinations or under conditions of continuous absorption for varying periods of time. Thus, late in 1900 and early in 1901, it was observed that in parts of northern England cases of peripheral neuritis were becoming increasingly numerous, particularly among beer drinkers. At first this was attributed to alcoholism, but as cases were found to have occurred among moderate drinkers attention was directed to other possible causes, and late in 1901 analysis of samples of beer revealed the presence of arsenic in significant, though minute, amounts. A Royal Commission was appointed and the occurrence critically investigated, together with the related subject of arsenical poisoning, and in 1903 a full and detailed Report was published. In concluding the Report, the recommendation was made that solid foods and foodstuffs should not be contaminated with arsenic to an extent greater than 1/100th of a grain per pound. This recommendation is now forty years' old and has been generally accepted and stood the test of time in a remarkable fashion, yet it can still be stated authoritatively that more precise knowledge is required of the cumulative effect of repeated minute doses of arsenic, and that the relative toxicity of inorganic forms of arsenic and organic forms (such as occur in shell-fish) is still more or less unknown. Again, the toxicity of lead has probably been the subject of more investigation than that of any other metal, and by common consent a maximum safe limit has been accepted, yet the medical data of men working in lead industries frequently suggest that many of them must regularly absorb more than this maximum. Tolerance and idiosyncrasy confuse the issue, the former to a greater extent because it may characterise a group, whereas the latter is essentially individualistic. The real difficulty arises, however, from the comparative absence of data which are indispensible, namely, the results of direct experiments on human beings on a sufficiently comprehensive scale. To correlate animal experiments with human requirements is of doubtful value, particularly as a substantial proportion of such experimental work has been made by the administration of inorganic salts, either in solution or mixed with food but not, as far as our knowledge goes, in the forms in which the elements are likely to be present in foods. The requirement for direct experiments with human beings is impracticable and incapable of fulfilment. It remains therefore to seek an answer—even if only a tentative one—in another way. What amounts of these elements are daily ingested in food by the average person; what would be the expected effect on the health of the average person; and what medical evidence is there of significant or potential variations in average health? An example of such a method of attack is afforded by the work that led to goitre prophylaxis with iodine.

Dr. Monier-Williams's book is probably the first published attempt to survey critically the whole field, and, as would be expected from such an authority, it is a great achievement. It deals with 26 elements in separate chapters, a single chapter is devoted to barium and strontium, and the last chapter (of 7 pages) under the heading of "Lithium and Other Metals," contains references to lithium, beryllium, zirconium, cerium, germanium, tungsten, thallium and uranium. Each element is examined from all angles and the information goes far beyond what might be expected from the title: the occurrence and amounts in different foods, the biological functions of the element, its toxic amount and effect, its retention in or method of excretion from the animal system, and the analytical method of determination (in outline but not in working detail) are all dealt with, and of course a very full bibliography is given. Better perhaps than a mere catalogue is the following quotation of the sub-headings in the longest chapter (Copper—64 pages).

Copper—As an essential element in plant and animal life; content of copper in organs of the body; biochemical availability; absorption, excretion and retention; minimum requirements; deficiency effects in cattle and sheep; pigmentation; toxicity; in soils and plants; copper fungicides; corrosion by foods; in dairy products; copper and vitamin C; copper and fermentation; greening of vegetables; copper haze in wines; copper in tomatoes; copper in various foods; copper and public health; the determination of copper. The bibliography covers 8 pages.

On similar lines lead takes 43 pages; zinc, 31; tin, 24; arsenic, 45; antimony, 15; cobalt, 12; manganese, 19; iodine, 19; selenium, 16; iron, 33; fluorine, 32; boron, 16; chromium, 5; molybdenum, 7.

With the aid of Dr. Monier-Williams's work the foundations of future legal limits for the metallic contamination of foodstuffs will be laid, and all workers in this field will not only congratulate him, but will also thank him very sincerely for its publication.

GEORGE TAYLOR

POLAROGRAPHISCHES PRAKTIKUM. By Professor J. HEYROVSKÝ. Anleitungem für die Chemische Laboratoriumspraxis. Band IV. Pp. vi + 118 with 90 Figures. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1948. Price DM 8.40.

The appearance of a new book on Polarography by Professor Heyrovský will be welcomed by many members of the Society who have met the distinguished founder of the subject, heard him lecture, or read his lecture in *The Analyst*. This monograph, as its title suggests, is a purely practical introduction to the subject. Those desiring information on the theory or wishing for an exhaustive compendium of methods and applications must look elsewhere. A distinct advantage of the present monograph is that it brings to one's notice numerous new practical devices in use in the Czechoslovakian school. English, and particularly American, practice is almost ignored. The pen recorders, so common in England and the U.S.A., are brushed aside as being only fit for "technical analysis." An excellent chapter on potential - time curves obtained by the oscillographic method may help to bring to a wider circle of polarographic workers the value of this method; the original paper, which appeared in the *Zeitschrift für physikalische Chemie* in 1943, has not been widely read in this country. An increase in sensitivity would make the oscillographic method quite important, but, even in its present state of development, it can give valuable information about the nature of electrode reactions.

Polarographisches Praktikum is excellently printed on good paper and the many illustrations are well produced. The paper binding is poor. W. CULE DAVIES

British Standards Institution

NEW SPECIFICATIONS*

B.S. 391: 1948.	Tung Oil (Types F and M). Price 2s. 6d.
B.S. 522: 1948.	Ortho, Meta and Para Cresols. Price 2s.
B.S. 523: 1948.	Phenol. Price 2s.
B.S. 524: 1948.	Refined Cresylic Acid. Price 2s. 6d.
B.S. 1416: 1948.	Sampling and Analysis of Rennet Casein. Price 2s. 6d.
B.S. 1917: 1948.	Sampling and Analysis of Acid Casein. Price 2s. 6d.
B.S. 1482: 1948.	Home-produced Technical Tallow. Price 2s. 6d.
B.S. 1983: 1948.	Home-produced Technical Bone Grease. Price 2s. 6d.
B.S. 1499: 1949.	General Guidance in Sampling Non-ferrous Metals. Price 1s.

A FEW copies of the following draft specification, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, J. H. Lane, 7-8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel—

CK(ISE)1883 (To supersede CK(ISE)635)—Method for the Determination of Aluminium Oxide in Permanent Magnet Alloys.

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* Obtainable from the British Standards Institution, Publications Department, 28, Victoria Street, London, S.W.1.

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m., on Wednesday, April 6th, at Gas Industry House, 1 Grosvenor Place, London, S.W.1. The Chair was taken by the President, Mr. George Taylor. The following papers were presented and discussed: "A Technique for Paper Chromatography, using Volatile Solvents, with Special Reference to the Estimation of Penicillin in a Mixture," by P. B. Baker, M.P.S., F. Dobson and A. J. P. Martin, M.A., Ph.D.; "Penicillin, some Applications of a Chromatographic Technique in Production Control," by J. W. Albans, B.Sc., A.R.I.C.; "Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin. Part 1. The Determination of Benzyl Penicillin by Precipitation with N-Ethylpiperidine," read by C. R. Bond, M.Sc., F.R.I.C., Chairman of the Sub-Committee.

NEW MEMBERS

John Andrews, jun.; John Anthony; David Baird, F.R.I.C.; Geoffrey Robert Ball, B.Sc. (Lond.); Edward Gordon Brown, A.M.C.T., A.R.I.C.; George Bremner Cochrane; Leslie Schofield Crabtree; David Robert Davies, B.Sc. (Lond.), A.R.I.C.; Robert Edward Leo Davies, B.Sc. (Liv.), A.R.I.C.; Ronald Ernest Hack, Ph.C.; Alan Hutchinson; Harold Jackson, A.R.I.C., A.M.C.T.; Edwin Hart Nurse, B.Sc. (Lond.), F.R.I.C.; Victor Henry Parker, B.Sc. (Lond.); Reginald Oliver Samuel, B.Sc. (Lond.), A.R.I.C.; James Shaw; Samuel Campbell Sloan; Dennis George Stringer, A.M.Inst.GasE.; Norman Darby Sylvester, M.Sc. (Birm.), F.R.I.C.; Miss Barbara Seaton Winton, B.A. (Cantab.).

PHYSICAL METHODS GROUP

THE Twenty-first Ordinary Meeting of the Group was held at 3.30 p.m. on Friday, April 1st, 1949, in the Chemistry Lecture Theatre, The University, Nottingham. Dr. J. G. A. Griffiths was in the Chair, and about 60 members and visitors were present.

The following papers on Electrophoretic Analysis were read and discussed before tea: "The Technique of Moving Boundary Electrophoresis," by R. A. Kekwick, D.Sc.; "Electrophoresis in the Analysis of Serum Proteins," by N. H. Martin, M.A., M.B., M.R.C.P., F.R.I.C.

During the tea interval a Tiselius electrophoresis apparatus was demonstrated by Miss M. Meiklejohn, B.Sc., and Mr. H. E. Brookes, B.Sc.

After tea, the following papers were read and discussed: "The Effect of Antrypol on the Electrophoretic Pattern of Sera," by A. E. Ambler, B.Sc., and J. Madinaveitia, Ph.D.; "Ionophoresis of Amino Acids and Peptides," by A. J. P. Martin, M.A., Ph.D.

BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Biological Methods Group and the Agriculture Group of the Society of Chemical Industry was held on Tuesday, February 15th, 1949, in the Chemistry Department of the Royal College of Science, Imperial Institute Road, London, S.W.7, at 2.30 p.m.

The following papers were presented and discussed: "Introduction to the Evaluation of Selective Weedkillers," by M. A. H. Tincker, M.A., D.Sc., F.L.S., North of Scotland College of Agriculture, Aberdeen; "Laboratory Methods and Trials leading up to the Field Use of Selective Weedkillers," by W. G. Templeman, Ph.D., M.Sc., F.R.I.C., Jealott's Hill Research Station, Bracknell, Berks.; "The Evaluation of Selective Phytocidal Action in Field Trials," by E. K. Woodford, Ph.D., Department of Agriculture, University of Oxford; "The Determination of Selective Weedkillers in Soils, etc.," by J. H. Hamence, Ph.D., M.Sc., F.R.I.C., Analytical Laboratory, Peek House, Eastcheap, London, E.C.3.

The Analysis of Nylon and Related Polymers

By M. CLASPER and J. HASLAM

(*Read at the Meeting of the Society on Wednesday, February 2nd,* 1949)

In the past two years several samples of nitrogen-containing polymers that appeared to be of nylon type have been received in our laboratory for examination. They presented considerable difficulty because determination of the physical constants and certain chemical data did not yield conclusive information as to the full nature of the samples. For this reason it was considered desirable to undertake a rather detailed analytical examination of 66 nylon and related polymers in order to have available fundamental information that would be of value in assessing the various samples submitted to us.

The literature on the analysis of nylons is rather scanty.

Shinkle[1] gives information about the identification of nylon, based on its behaviour towards solvents such as phenol and its appearance under the microscope with and without polarised light.

Hart[2] suggests that a procedure that he worked out for the analysis of cationic surface-active agents of the tervalent nitrogen type, involving the determination of the acid used to render the products soluble, nitrogen as alkalinity, total active ingredients or amido-amine, free fatty acids, total fatty acids, condensed amine and uncondensed amine, might be applicable to the analysis of nylon.

Gerber and Lathrop,[3] in a paper on "Qualitative Identification and Quantitative Estimation of Nylon in the presence of Silk, Wool and Cotton," suggest that nylon in undyed fibre mixtures is most readily distinguished by dyeing with neocarmine. They describe procedures for the separation of nylon from cotton, silk, etc., based on the behaviour of the fibres towards solvents such as acetic acid, 5 per cent. potassium hydroxide solution, acetone and calcium thiocyanate solution.

Freedman and Nechamkin[4] separate nylon from other fibres by treatment with a mixture of 60 ml. of concentrated hydrochloric acid and 40 ml. of water at 38° C.

Saburada and Hizawa,[5] as a result of X-ray and analytical examination of du Pont's nylon, suggest that of the 12 carbon atoms, 6 belong to the dicarbonic acid and 6 to the diamine, and they indicate that their hydrolysis of nylon proved that it is composed of adipic acid and hexamethylene diamine.

Miller, Mack and Chapman[6] compare the microscopical characters of nylon and lanital textile fibres and the behaviour of the two fibres towards chemical reagents such as Millon's reagent and cuprammonium solution, to solvents such as acetone and phenol and to staining reagents such as neocarmine W.

A summary report[7] on the work of Technical Committee C of the Unification of Testing Methods Committee outlines chemical, physical and microscopic tests for identifying animal, vegetable and manufactured fibres, including casein, vinyon, nylon and alginate fibres.

Hosino[8] suggests that nylon, after having been washed with ether, is almost completely hydrolysed to adipic acid and hexamethylene diamine by heating with concentrated hydrochloric acid for 12 hours.

Nechamkin[9] describes a schematic procedure depending on burning test pieces and noting the odour and appearance of the flame and residue for the identification of various resins, including nylon.

Howlett, Morby and Urquhart,[10] in a comprehensive paper, describe methods for the analysis of fibre mixtures, including mixtures containing nylon. The methods are based essentially on differential solubility in acids, alkalis, salt solution and solvents.

Romeo,[11] in a paper dealing with the microscopic and analytical characteristics of nylon, points out that it can be separated from natural and synthetic cellulose fibres by its behaviour towards sulphuric acid, from cotton by its reaction with acetic acid and from silk and rayon by its reaction with calcium thiocyanate.

Algerino[12] describes procedures for the systematic identification of textile fibres, including nylon, based on the different behaviour of the fibres in solvents and solutions of inorganic salts.

Eyre[13] has carried out work on similar lines to Algerino.[12]

Kappelmeier and van Goor[14] describe a method by which polyamide resins may be identified by examination of the products obtained on acid hydrolysis.

EXPERIMENTAL

HYDROLYSIS OF 66 NYLON—

The original experiments on the hydrolysis of 66 nylon were carried out by heating 1 g. of the sample with 25 ml. of 50 per cent. v/v sulphuric acid solution under reflux. Although solution of the sample was complete after 15 minutes heating, progressive darkening of the solution took place subsequently. The resulting solution was distilled in steam, but no acid was found in the steam distillate. Simple ether extraction of the hydrolysis product yielded adipic acid of high purity as shown by determination of equivalent weight, although the yield was very low, *viz.*, 0·04 g. compared with a theoretical yield of 0·646 g.

In view of the unsatisfactory results obtained on hydrolysis with 50 per cent. v/v sulphuric acid, experiments were made on hydrolysis of 66 nylon with 20 per cent. v/v hydrochloric acid (*i.e.*, 1 vol. of concentrated hydrochloric acid, sp.gr. 1·16, diluted to 5 vol. with water). After heating 1 g. of the sample for 22 hours under reflux, the hydrolysis products were extracted with ether and the ethereal solution was washed with water and evaporated, and the residue dried at 100° C. and weighed. Although the recovered acid possessed an equivalent weight of 71·5 (theory for pure adipic acid 73), the yield only amounted to 8 per cent. of the theoretical amount.

EXTRACTION OF ADIPIC ACID FROM HYDROLYSIS PRODUCTS—

To test the efficiency of the ether extraction procedure, 1·6 g. of a mixture of known amounts of hexamethylene diamine and adipic acid was heated with 20 ml. of 20 per cent. v/v hydrochloric acid until complete solution of the adipic acid was effected. The solution was cooled and extracted in a separating funnel with ether. The combined ether extracts were washed with water to remove traces of chloride, but the recovered adipic acid only amounted to 16·2 per cent. of the amount added. Subsequent extraction with ether, in a continuous liquid/liquid extractor, of the above ether-extracted solution, and final washing with water of the ether extract obtained yielded a further 20·7 per cent. of adipic acid, making a total of 36·9 per cent. of the amount added.

The experiment was repeated with extraction in a continuous liquid/liquid extractor, but this time the ether extract was evaporated to dryness without previous washing with water to remove hydrochloric acid. With 4-hour extraction the adipic acid recovered after drying at 100° C. amounted to 96 per cent. of that taken and further extraction for 4 hours yielded an additional 4·4 per cent., making a total of 100·4 per cent. It was then shown that extraction for 6 hours of a solution of 1 g. of a mixture of equivalent proportions of adipic acid and hexamethylene diamine in 20 ml. of 20 per cent. v/v hydrochloric acid in the above manner gave recoveries of 99·5, 99·9 and 97·7 per cent. of the adipic acid added. The equivalent weights of the three specimens recovered were respectively 73·5, 73·1 and 72·9.

RECOVERY OF HEXAMETHYLENE DIAMINE FROM HYDROLYSIS PRODUCTS—

The recovery of hexamethylene diamine after hydrolysis of 66 nylon salt was now considered.

A sample of 66 nylon salt, *i.e.*, the salt obtained by combination of molecular proportions of hexamethylene diamine and adipic acid, was hydrolysed by boiling with 20 per cent. v/v hydrochloric acid solution as outlined on page 000. After extraction of the adipic acid the extracted aqueous solution was made alkaline with dilute sodium hydroxide solution and extracted with ether in a continuous liquid/liquid extractor for 6 hours. The ether extract was evaporated and dried at 100° C., but the residue obtained was exceedingly hygroscopic, difficult to weigh and tended to decompose, turning brown. Accordingly, this residue was titrated with 0·1 *N* hydrochloric acid, and the neutralised solution was evaporated to dryness in the water-bath and finally dried to constant weight at 100° C. The recovery of hexamethylene diamine was low compared with the amount anticipated from the theoretical composition of 66 nylon salt. Calculated from the weight of hydrochloride the recovery of hexamethylene diamine was 73·0 per cent. of theory, whereas calculated from the titration of the base, it was 68·1 per cent. of theory.

Application of the above method of extraction to solutions of hexamethylene diamine, the strength of which had previously been determined by titration, yielded amounts of hexamethylene diamine in the ether extract equivalent to 62 to 86 per cent. when calculated from the titration of the ether extract and 78 to 91 per cent. when calculated from the weight of hydrochloride. Further strong evidence was obtained that the hydrochloride was not very pure, and that it was contaminated with alkali presumably derived from that used in rendering the solution alkaline before ether extraction.

The recoveries of hexamethylene diamine by extraction of alkalised solution were obviously unsatisfactory, and it was decided to simplify the procedure considerably by carrying out hydrolysis of nylon samples with acid, extracting the liberated organic acid and then evaporating the solution of the residual hydrochlorides directly to dryness. In addition, an improved form of liquid/liquid extractor was introduced, full dimensions of which are given in Fig. 1.

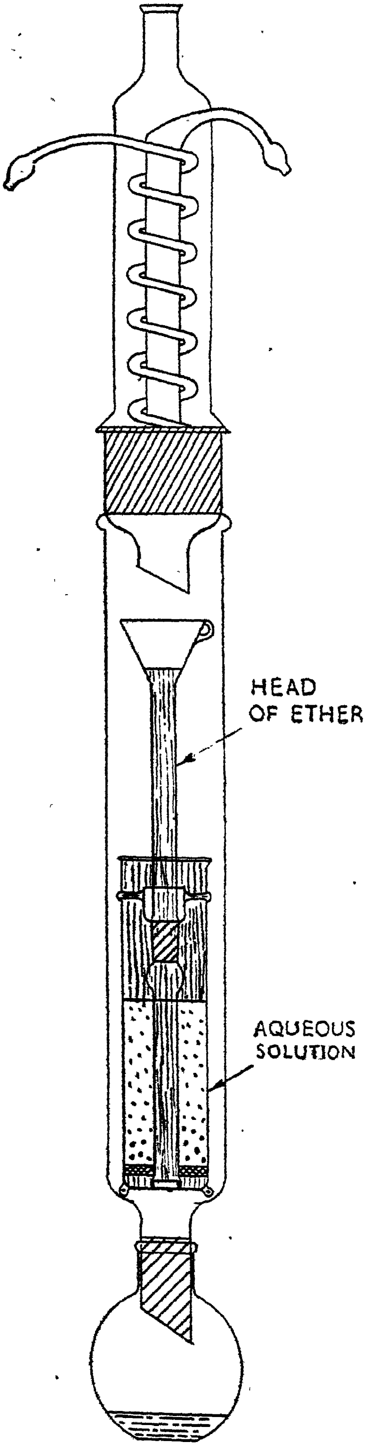


Fig. 1a. Modified continuous liquid/liquid extractor

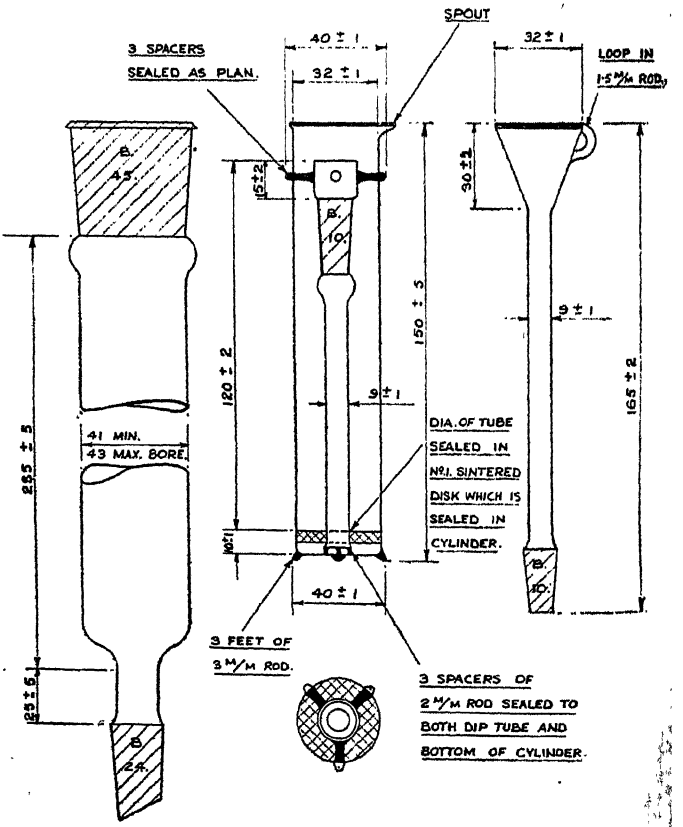


Fig. 1b. Modified continuous liquid/liquid extractor
Dimensions are in millimetres

This modified procedure was applied satisfactorily to the examination of bright and delustred 66 nylon polymer. One gram of the sample was hydrolysed by boiling for 20 hours with 20 ml. of 20 per cent. v/v hydrochloric acid solution. The hydrolysis product was extracted for 6 hours with ether and the recovered extract evaporated, dried at 100° C. and weighed. The residual solution of the hydrochloride was evaporated to dryness, dried at 100° C. and weighed. Certain other figures were determined, such as the melting-point and the equivalent of the organic acid, and the melting-point (Mason's method) and the chlorine content (by electrometric titration) of the hydrochloride. The results obtained are given in Table I.

TABLE I

ANALYSES OF BRIGHT AND DELUSTRED 66 NYLON POLYMER

Sample	Ether extract of hydrolysed products. *Acid* %	M.p. of acid ° C.	Equivalent weight of acid	Residue after evaporation of ether-extracted hydrolysed products. *Base hydrochloride* %	M.p. of base hydrochloride ° C.	Chlorine content of base hydrochloride %
Bright polymer	63·1	149·5	73·6	81·0	251·0	37·2
	62·9	149·5	74·1	80·6	251·0	37·2
Delustred polymer	62·8	148·5	73·9	80·9	251·5	37·1
	63·1	149·0	74·1	81·6	251·0	37·1
	63·3	149·0	74·5	81·0	251·0	37·3

Note 1—In the case of the delustred polymer, the hydrolysed solution was filtered before the extraction of the adipic acid, and the insoluble matter, principally TiO_2, was washed, dried and weighed. It amounted to 0·31 per cent.

Note 2—The theoretical figures for the nylon produced by the union of 1 molecule of adipic acid and 1 molecule of hexamethylene diamine, with elimination of 2 molecules of water, are adipic acid 64·6 per cent. and hexamethylene diamine hydrochloride 83·6 per cent. The accepted values for the other data are:

Melting-point of adipic acid, 150° C.
Equivalent weight of adipic acid, 73.
Melting-point of hexamethylene diamine hydrochloride, 253° C.
Chlorine content of „ „ „ 37·5 per cent.

APPLICATION OF METHODS TO 610 NYLON FILAMENT AND CHIPS—

Attempts to apply the above method to the hydrolysis of 610 nylon filament were at first unsuccessful. Even after 40 hours the hydrolysis was incomplete. The proportion of recovered acid was low, *viz.*, 65 per cent., and the hexamethylene diamine hydrochloride was impure (chlorine content = 35 per cent.), presumably owing to contamination with unhydrolysed nylon. There was also trouble due to bumping during the hydrolysis and, in addition, sebacic acid was present in the hydrolysis product, essentially in suspension but part in solution.

The method was also unsuccessful when applied to 610 nylon chips. The hydrolysis products on filtration left behind a semi-gelatinous mass which was extremely difficult to filter and contained some sebacic acid, difficult to extract. The filtrate contained some sebacic acid in solution and after extraction of this the evaporated solution of the hydrochloride left a residue that obviously contained some unhydrolysed sample, for its chlorine content was only 35 per cent. and it melted indefinitely at 242° C. These figures were raised to 37 per cent. and 253° C. on re-crystallisation.

It was concluded that the samples we were using were not in a sufficiently fine state of subdivision to enable us to achieve complete hydrolysis. Accordingly a sample of 610 nylon chips was dissolved in *m*-cresol and the polymer was reprecipitated from the solution in a very finely divided condition, by addition of methanol in the following way.

About 5 g. of the 610 nylon chips were dissolved by standing for approximately 24 hours in 30 to 40 ml. of *m*-cresol. The nylon polymer was then precipitated by slowly adding methanol, with constant stirring, until the precipitation was complete. About 100 ml. of methanol were required. The precipitate was filtered off, collected in a beaker and washed by swirling with 100 ml. of methanol and re-filtering. This washing was repeated with a further lot of methanol followed by three 100-ml. lots of ether to ensure removal of *m*-cresol. The finely divided nylon polymer thus obtained was exposed to the atmosphere overnight to remove the last traces of ether and finally dried in an oven at 100° C.

One gram of the finely divided material was heated under reflux for 40 hours with 20 ml. of 20 per cent. v/v hydrochloric acid. The warm hydrolysis product was filtered through a No. 1G3 sintered-glass crucible and the insoluble residue on the filter washed with a small amount of water, dried in the oven at 100° C. and extracted with ether in a modified Wiley extraction apparatus. The filtrate and washings were evaporated on the hot-plate to about 25 ml. and then extracted with ether in the continuous ether-extraction apparatus. The insoluble matter in the sintered-glass crucible was dried and weighed to yield the amount of unhydrolysed material, whilst the two ether extracts were combined, evaporated, dried and weighed. The solution of the base hydrochloride was evaporated and dried as usual. The results given in Table II were obtained on examination of an authentic 610 nylon.

TABLE II

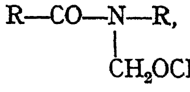
ANALYSIS OF 610 NYLON POLYMER

Test	Unhydrolysed matter %	Ether extract of hydrolysed products. *Acid* %	M.p. of acid ° C.	Equivalent weights of acid	Residue after evaporation of ether-extracted hydrolysed products. *Base hydrochloride* %	M.p. of base hydrochloride ° C.	Chlorine content of base hydrochloride %
1	0·32	71·1	132	103·5	65·8	250	36·9
2	0·36	69·5	132	102·9	67·3	252	37·1

APPLICATION TO AUTHENTIC SAMPLES OF VARIOUS NYLONS AND RELATED POLYMERS—

At this stage we secured through the courtesy of Dr. Heyes of our Technical Service Department authentic samples of nylon and related polymers. Their descriptions were as follows:—

(1) A sample of 6 nylon polymer prepared in the laboratory.
(2) A sample of 66 nylon polymer.
(3) An interpolymer of 66 nylon and 610 nylon, the proportions of which were unknown.
(4) An interpolymer of 66 nylon and 6 nylon in the proportion of 95 : 5.
(5) A sample of 610 nylon polymer.
(6) An interpolymer of 66 nylon, 610 nylon and 6 nylon in the proportions of 40 : 30 : 30.
(7) An interpolymer of 66 nylon and 6 nylon in which the proportions were unknown.
(8) A sample of 66 nylon polymer which had been modified by treatment with formaldehyde and methanol. This treatment introduces methoxymethyl groups probably as follows—



$$\begin{array}{c} R\text{—}CO\text{—}\underset{\displaystyle CH_2OCH_3}{\underset{|}{N}}\text{—}R, \end{array}$$

and renders the nylon polymer soluble in alcohol.

These samples were examined by the methods we have outlined, with hydrolysis for 40 hours. The results are set out in Table III, and for easy comparison the figures obtained on the 610 nylon sample given in Table II are included. This was the only sample requiring reprecipitation from *m*-cresol solution. Certain other figures were obtained on these authentic samples, *viz.*, melting-points, and carbon, hydrogen and nitrogen contents by macro-methods. For the nitrogen determinations 0·25 g. of the nylon polymer was taken and digested for 5 hours, with 7 g. of sodium sulphate/selenium (35 : 1) catalyst and 20 ml. of concentrated sulphuric acid. It may be mentioned here that nitrogen determinations in triplicate on 0·05 g. of a sample of 66 nylon by Ling's method[16] gave the results 11·87, 11·76 and 12·00 per cent.

It is suggested that in the case of nylon 6 the base hydrochloride is weighed as $HClNH_2(CH_2)_5COOH$. With all the other samples, except the two 66/6 polymers, the

TABLE III

ANALYTICAL RESULTS OBTAINED ON NYLON AND RELATED POLYMERS

Sample	Un-hydrolysed matter %	Ether extract of hydrolysed products. *Acid* %	Residue after evaporation of ether-extracted hydrolysis products. *Base hydro-chloride* %	M.p. of acid ° C.	Equivalent weight of acid	M.p. of base hydro-chloride ° C.	Chlorine in base hydro-chloride %	Chlorine in base hydro-chloride as percentage of sample taken	Nitrogen content of sample %	Carbon content of sample %	Hydrogen content of sample %	M.p. of sample ° C.
Nylon 6	0·43	2·2	139·6	—	—	122	20·9	29·3	11·69	61·2	10·0	217
(caprolactam)	0·88	2·9	137·8	—	—	125	20·8	28·9	11·65	61·4	10·1	217
Nylon 66	nil	62·1	81·3	148	73·7	252	37·2	30·3	11·71	61·0	9·9	256
	nil	61·8	82·3	148	74·5	253	37·3	30·7	11·75	61·2	9·9	255
Nylon 610	0·32	71·1	65·8	132	103·5	250	36·9	24·3	9·52	66·2	10·8	220
	0·36	69·5	67·3	132	102·9	252	37·1	24·9	9·53	66·2	10·8	219
Nylon 66/6 (95 : 5)	nil	56·0	85·9	150	73·0	210–220*	34·4	29·5	11·74	60·9	10·0	246
	nil	55·6	85·9	150	73·4	213–218*	34·3	29·4	11·77	61·0	9·9	246
Nylon 66/6 (proportions unknown)	0·02	36·7	104·8	150	73·0	119–142†	28·1	29·4	11·71	60·8	10·0	185–190
	0·12	36·0	104·8	149	72·8	116–138†	29·1	29·5	11·75	60·9	10·0	186–190
Nylon 66/610	nil	61·7	82·3	140–144*	75·8	254	37·0	30·5	11·73	61·9	10·0	251–254
	nil	62·3	81·9	142–146*	75·5	254	37·0	30·5	11·73	61·9	10·0	253
Nylon 66/610/6 (40 : 30 : 30)	nil	45·3	94·7	120	83·5	116–118	29·6	27·9	11·07	62·6	10·2	168–172
	nil	—	94·2	122	84·0	116–118	29·6	28·1	11·09	62·4	10·2	167–170
Nylon 66 (methoxymethyl substituted)	0·24	52·0	69·9	149	73·6	250	36·8	25·7	10·14	59·9	9·9	180–190†
	0·21	52·5	70·1	150	73·2	251	36·6	25·7	10·16	59·8	9·9	185–195†

* M.p. indefinite. † M.p. very indefinite.

base hydrochloride is the dihydrochloride of hexamethylene diamine. With the 66/6 polymers the base hydrochloride consists of a mixture of the dihydrochloride of hexamethylene diamine and $HClNH_2(CH_2)_5COOH$.

Further observations on the results shown in Table III will be found on page 229.

SEPARATION OF SEBACIC ACID FROM ADIPIC ACID—

The methods we had worked out apparently enabled us to effect complete breakdown of the samples but it was necessary to extend the work to the resolution of co-polymers, and this involved the separation of mixed acids and mixed base hydrochlorides. Attention was first directed to the separation of adipic and sebacic acids.

Attempts to separate sebacic acid from adipic acid in hydrochloric acid solution by differential extraction with ether or by steam distillation were unsuccessful.

The behaviour of neutral adipate and sebacate solutions on treatment with solutions of metallic salts was next investigated. 0·5 g. of the respective acid in water was made alkaline with ammonia solution and the excess of ammonia boiled off. The residual solution was filtered and the filtrate made up to 50 ml. The sebacate solution was slightly turbid. To 5 ml. of the prepared solution was added 1 ml. of the following solutions, with the results shown.

Solution	Adipic acid	Sebacic acid
Silver nitrate, 10% ..	Flocculent precipitate	Flocculent precipitate
Copper sulphate, 5%	Precipitate develops on standing	Fairly crystalline precipitate
Zinc sulphate, 5%	No precipitate	Crystalline precipitate
Calcium chloride, 5%	No precipitate	Slight crystalline precipitate
Ferric chloride, 10%	No precipitate	Gelatinous precipitate

It appeared that a separation of the two acids might be possible by using zinc sulphate as precipitant. A prepared mixture containing adipic acid (0·504 g.) and sebacic acid (0·054 g.) was treated with ammonia solution, the excess of ammonia was boiled off and the solution cooled and treated with 5 per cent. zinc sulphate solution. The precipitate was coagulated by warming and then filtered, washed with water, dried and weighed. The recovered zinc derivative weighed 0·565 g., which pointed to serious contamination of the zinc sebacate with zinc adipate.

Chromatographic separation of the two acids did not appear to be promising.

At this stage an attempt was made to develop a method for the separation of adipic and sebacic acids along the lines suggested by Brancker[15] in his paper on the "Stoichiometric Extraction of Mixed Acids." Mixtures of adipic and sebacic acids were placed in contact with water and an immiscible solvent. *N* Sodium hydroxide was added, 0·2 ml. at a time, with shaking, until an amount of alkali equivalent to the adipic acid present had been added. The solvent layer was then separated and evaporated and the equivalent of the recovered acid determined. With benzene, chloroform or carbon tetrachloride as immiscible solvent the recovery of acid from the solvent layer was poor, but with ether, although the recovery was not quantitative, the acid possessed the correct equivalent for sebacic acid, *viz.*, 99·8 and 101·9.

In further experiments along these lines known mixtures of adipic and sebacic acids in a mixture of 50 ml. of water and 35 ml. of ether were treated with *N* sodium hydroxide until an amount equivalent to the adipic acid had been added. The ether layer was separated, evaporated, dried at 100° C. and weighed. From a mixture of 0·3537 g. of adipic and 0·1534 g. of sebacic acid, 0·0653 g. of acid was recovered of equivalent weight 105·3. The aqueous solution was then extracted in a continuous ether-extraction apparatus and yielded a further 0·0741 g. of acid of equivalent weight 107·7. The residual liquor, made acid with hydrochloric acid and extracted with ether, yielded an ether extract weighing 0·3689 g., of equivalent weight 80·0. A repeat experiment gave comparable figures. These experiments suggested that the separation of adipic and sebacic acids was almost complete, although there was evidence that the recovered acids contained sodium salts as impurity.

When a mixture of 0·3520 g. of adipic and 0·1515 g. of sebacic acid was treated with alkali equivalent to the adipic acid and the whole extracted in a continuous ether-extraction apparatus the recovered acid weighed 0·1457 g. and had equivalent weight 99·5, and m.p. 130·5° C. The solution was then acidified with hydrochloric acid and extracted with ether; the extract, dried at 100° C., weighed 0·3544 g. and had equivalent weight 76·8 and m.p. 144° to 147° C. The rather indefinite melting-point and high equivalent weight pointed to the presence of sodium salts in this acid.

The above experiments seemed to indicate that it might be possible to determine adipic and sebacic acids in the presence of one another by equilibrating the mixed acids with known volumes of water and ether at a definite temperature, and then titrating an aliquot of the aqueous solution with standard alkali. Preliminary experiments in separating funnels were successful.

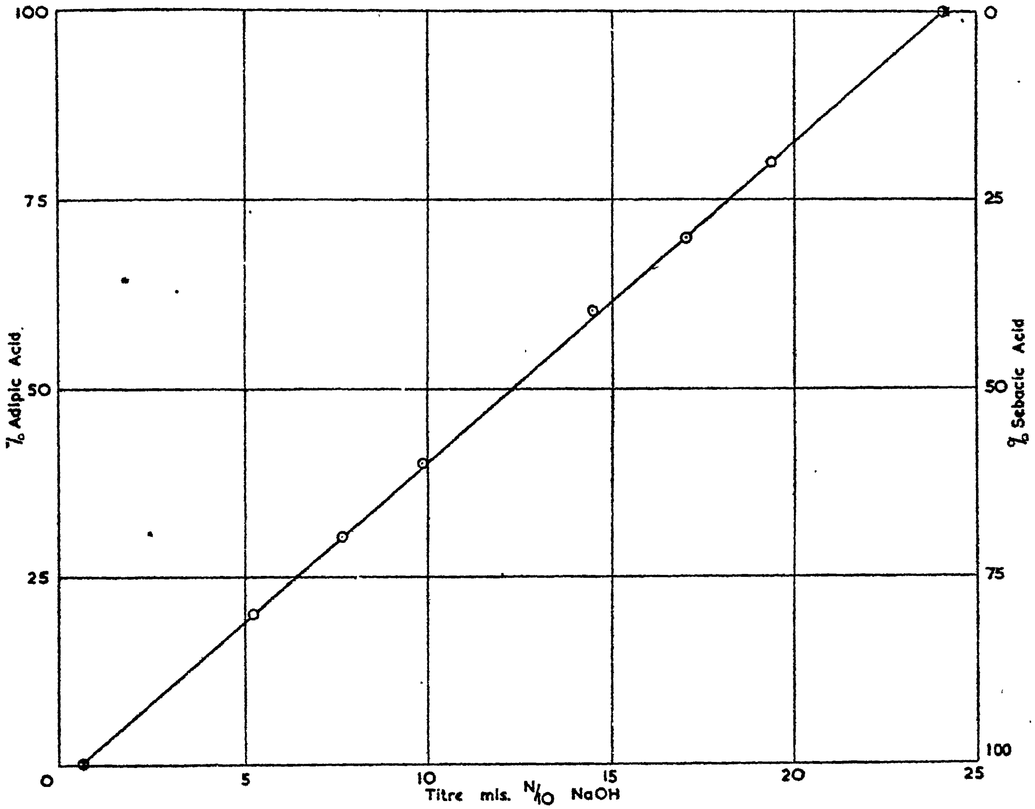


Fig. 2. Graph for adipic acid - sebacic acid mixtures

At this stage we became aware of the fact that the equivalent weights of adipic and sebacic acids used were slightly high, but it appeared from the literature that previous workers on adipic acid had used acid dried at 120° to 130° C. in work involving adipic acid as an acidimetric standard. Accordingly, recrystallised acids were first dried at 120° C. and their equivalent weights found to be 73·3 for the adipic acid and 101·9 for the sebacic acid. Known mixtures of these two recrystallised acids were then taken (total weight 0·5 g.) and equilibrated in stoppered bottles with 50 ml. of water and 35 ml. of ether at 20° C. for 30 minutes after which 25 ml. of the aqueous layer were withdrawn and titrated with 0·1 *N* sodium hydroxide in presence of phenolphthalein as indicator. Results obtained are shown in Table IV, and a graph was constructed on the basis of these results (Fig. 2).

TABLE IV

Adipic acid taken g.	Sebacic acid taken g.	Adipic acid in mixture %	0·1*N* NaOH titre for 25 ml. of aqueous layer
0·5001	nil	100	24·12
0·4000	0·1001	79·99	19·38
0·3501	0·1499	70·02	17·00
0·3001	0·2000	60·01	14·52
0·2002	0·3001	40·02	9·82
0·1498	0·3503	29·96	7·60
0·0999	0·3998	19·99	5·21
nil	0·5005	nil	0·71

The method was tested on known mixtures of adipic and sebacic acids, the composition of which was unknown to the operator at the time of test and the following results were obtained:—

Adipic acid present, %	Adipic acid found, %
60·9	60·7
32·1	31·6

Having proved that the two acids, adipic and sebacic, could be determined in presence of each other, attempts were made to devise a method that would yield the correct proportions of the two acids in a mixture and further establish their identity; this would be of great value in dealing with the products of hydrolysis of 66 and 610 nylon co polymers. Such a method was devised and its method of application to a mixture of adipic and sebacic acids is given below.

0·2501 g. of adipic acid and 0·2499 g. of sebacic acid were equilibrated in a glass-stoppered bottle at 20° C. for 30 minutes with 50 ml. of water and 35 ml. of ether. 25 ml. of the aqueous layer were withdrawn and titrated with *N* sodium hydroxide from a micro-burette in presence of 1 drop of phenolphthalein indicator. The volume required was 1·22 ml., indicating, according to the graph (Fig. 2), that the adipic acid content of the mixture was 49·6 per cent. (calc. 50·0 per cent.). This 49·6 per cent. of adipic acid was equivalent to 3·40 ml. of *N* sodium hydroxide. Accordingly, 3·40 − 1·22 = 2·18 ml. of *N* sodium hydroxide were added to the titrated solution, which was returned to the glass-stoppered bottle containing ether, etc., and the whole mixture shaken and transferred to a continuous liquid extractor. After 3 hours extraction with ether the ethereal solution was evaporated and dried at 120° C. and the melting-point and equivalent weight of the recovered acid were determined. The figures obtained were equivalent weight 103·2 and m.p. 130·5° C. The aqueous solution in the extraction apparatus was acidified with hydrochloric acid and extracted with ether in the continuous liquid/liquid extractor. The ethereal solution was evaporated to dryness and the residue recrystallised once from water and dried at 130° C. previous to determination of its melting-point and equivalent weight. The figures obtained on the recovered acid were m.p. 147° C. and equivalent weight 74·4.

This satisfactory resolution of a mixture of adipic and sebacic acids with recovery of satisfactory samples of the individual acids was confirmed by examination of an entirely different mixture of 0·4001 g. of adipic acid and 0·0999 g. of sebacic acid. After the initial equilibration the adipic acid calculated from the graph (Fig. 3) was 79·9 per cent. as against the calculated value of 80·0 per cent. The recovered acids had the following characters.

	Melting-point ° C.	Equivalent weight
Adipic acid	147·0	74·8
Sebacic acid	130·5	103·5

OBSERVATIONS ON THE ANALYTICAL RESULTS OBTAINED ON NYLON AND RELATED POLYMERS (SEE TABLE III)

NYLON 66—

In our experience, 66 nylon melts at 255° to 256° C. and gives analytical figures for nitrogen and carbon that are below theory but reproducible. We suspect that the elimination of water in the synthesis of 66 nylon is not as complete as is generally assumed. Nylon 66 is comparatively readily hydrolysed, without leaving any insoluble residue. The proportion of adipic acid recovered, *viz.*, 62 per cent., is very characteristic although below theory, as is the proportion of base hydrochloride, *viz.*, 82 per cent. The properties of the recovered acid and base hydrochloride are consistent with what one might expect from the nature of the polymer.

NYLON 610—

Nylon 610 melts at 219° to 220° C. The carbon and nitrogen figures are below theory but of course very different from those for the 66 nylon and therefore serve as very useful pointers to the type of nylon. The hydrolysis of 610 nylon is difficult and can usually only be satisfactorily accomplished after solution in *m*-cresol and reprecipitation. Even this reprecipitated material yields a small proportion of insoluble matter on hydrolysis. The proportion of sebacic acid recovered, *viz.*, 70 to 71 per cent., and base hydrochloride, *viz.*, 66 to 67 per cent., are in reasonably good agreement with theory, and the properties of each are consistent with the composition of the polymer.

NYLON 6 CAPROLACTAM—

Nylon 6 caprolactam polymer melts at 217° C. and the figures for carbon, hydrogen and nitrogen are in very good agreement with those for nylon 66 polymer, as might be expected on theoretical grounds. It is fairly readily hydrolysed, although a small amount of insoluble residue usually remains. The distinctive feature about nylon 6 polymer is the very low proportion of ether-soluble matter obtained after acid hydrolysis, *viz.*, 2·5 per cent., and the very large proportion of base hydrochloride, *viz.*, 138 to 140 per cent., of comparatively low chlorine content, *viz.*, 20·9 per cent., and low melting-point, 122° to 125° C.

NYLON 66/6 POLYMER—

Two samples of this polymer have been examined. One of these, said to be prepared in the proportion 95 of nylon 66 to 5 of nylon 6, possessed a melting-point slightly below that of nylon 66 polymer, but the carbon, hydrogen and nitrogen figures were in good agreement with those for this substance. This sample was hydrolysed comparatively readily, giving a proportion of recovered adipic acid, *viz.*, 56 per cent., below that from nylon 66 polymer and a percentage of base hydrochloride, *viz.*, 86 per cent., above that from nylon 66 polymer. The adipic acid was of high purity; the base hydrochloride was of rather indefinite melting-point, *viz.*, 210° to 220° C., but well below that of the base hydrochloride obtained from nylon 66 polymer. Furthermore, it contained only 34·3 per cent. of chlorine. The second sample of 66/6 polymer behaved in a way comparable to the above but obviously contained a much higher proportion of caprolactam. With this type of sample it would be useful to have available an accurate method of separating hexamethylene diamine hydrochloride and the hydrochloride of ε-amino caproic acid.

We have made tentative efforts to effect such a separation but, so far, without success. One method tried involved extraction of hexamethylene diamine at a high pH value followed by extraction of the ε-amino caproic acid at a lower one. A more promising method appeared to be that of a conductometric titration of the mixed hydrochlorides, because ε-amino caproic acid hydrochloride on conductometric titration with standard alkali gave two distinct breaks in the titration curve, the first of which corresponded with the neutralisation of the hydrochloric acid in the hydrochloride and the second corresponded with the total amino acid and hydrochloric acid. Perhaps the most promising line of attack on this problem may result from an observation made in the analysis of a nylon 66/6 polymer, from which it appears probable that by solution of the mixed hydrochlorides of hexamethylene diamine and ε-amino caproic acid in alcohol and precipitation with acetone almost pure hexamethylene diamine hydrochloride is precipitated. Further, we have evidence that the two hydrochlorides may be separated by the use of an ion-exchange resin.

NYLON 66/610 POLYMER—

The 66/610 polymer that we examined obviously contained a very small proportion of 610 polymer because the figures obtained were very comparable indeed with those for 66 polymer, with the possible exception of the melting-point of the recovered acid, 140° to 144° C. and the equivalent of this acid, *viz.*, 75·5. It is very probable that the application of the adipic acid - sebacic acid separation we have worked out would have brought out this point.

NYLON 66/610/6 POLYMER—

This sample had a low melting-point, *viz.*, 168° to 172° C. The nitrogen content (11·1 per cent.) was low, whilst the carbon content was rather high, *viz.*, 62·5 per cent., as compared with 66 nylon polymer. The sample was readily hydrolysed, the proportion of acid recovered was low, *viz.*, 45·3 per cent., whilst the proportion of base hydrochloride was high, *viz.*, 94·5 per cent. The base hydrochloride had a very low melting-point compared with 66 nylon polymer, whilst the chlorine content was only 29·6 per cent. These figures are consistent with the presence of amino caproic acid hydrochloride in the base hydrochloride. The recovered acid had a low melting-point, 120° C., and a high equivalent weight of 84; and application of the method worked out for the examination of mixtures of adipic and sebacic acids confirmed their presence and showed 43 per cent. of sebacic acid and 57 per cent. of adipic acid in the mixed acids. These figures were quite consistent with the presence of 30 per cent. of 610 polymer in the co-polymer and 40 per cent. of 66 polymer respectively.

NYLON 66 METHOXYMETHYL SUBSTITUTED—

This polymer had a rather lower carbon content than 66 nylon, *viz.*, 59·9 per cent., and a much lower nitrogen content, *viz.*, 10·1 per cent. It was readily hydrolysed, but the proportions of recovered acid, *viz.*, 52 per cent., and base hydrochloride, 70 per cent., were both lower than is usually found for 66 nylon. The recovered acid possessed the properties of adipic acid and the base hydrochloride those of hexamethylene diamine hydrochloride. This type of nylon readily yields formaldehyde on treatment with hydrochloric acid, and we found the apparatus illustrated in Fig. 3 very useful in the rapid detection of this type of polymer. The test was carried out as follows.

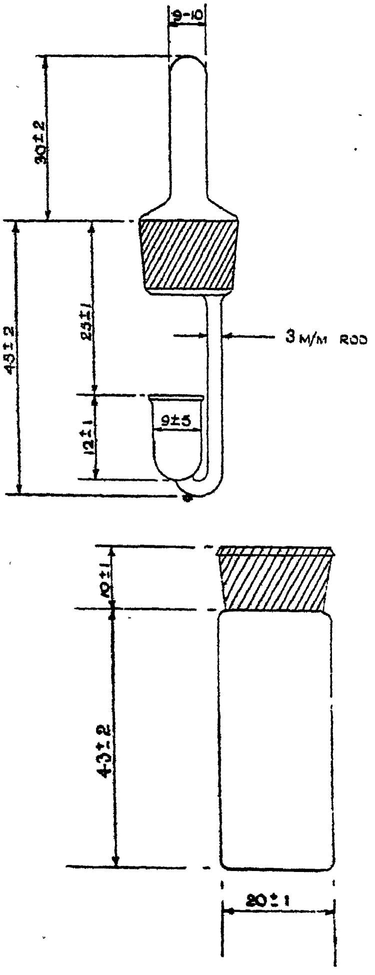


Fig. 3. Apparatus for the detection of volatile substances. Dimensions are in millimetres

A small amount of the nylon sample was placed at the bottom of the bottle and to this was added about 1 ml. of dilute hydrochloric acid. The stopper carrying the cup, which was half filled with decolorised Schiff's reagent, was then immediately inserted in position and the bottle placed in the dark and examined periodically. With formaldehyde-treated nylons the first trace of a violet coloration appeared after about 1 hour, and on allowing to stand overnight a deep violet colour was produced in the reagent. A control on ordinary 66 nylon and a blank were carried out alongside the test and no colour was obtained.

The presence of both free and combined formaldehyde in this type of sample may be shown by application of sulphite tests before and after hydrolysis, as follows:

Free formaldehyde—Weigh 3 g. of the nylon polymer into a 250-ml. ground-glass necked conical flask, add 75 ml. of ethyl alcohol and 25 ml. of water and place in a water-bath at 60° C. under a reflux condenser until solution occurs. Add 30 ml. of approximately N sodium sulphite solution with swirling and allow to remain at 60° C. for 90 minutes. Remove the flask, and titrate with 0·5 N hydrochloric acid, using thymolphthalein as indicator. From the titration obtained, corrected for a blank carried out on the reagents, calculate the percentage of formaldehyde from the following relationship.

Formaldehyde, % = ml. of 0·5 N HCl × 1·501/wt. of sample in grams.

Total formaldehyde—Weigh 3 g. of the nylon polymer into a 250-ml. ground-glass necked conical flask, add 80 ml. of water and 2 ml. of sulphuric acid and place in a water-bath at 60° C. under a reflux condenser for 2 hours. Withdraw the flask, add a few drops of thymolphthalein, neutralise the contents with N sodium hydroxide and then make just acid with 0·5 N hydrochloric acid. Add 50 ml. of approximately N sodium sulphite and after allowing to stand for 5 to 10 minutes titrate with 0·5 N hydrochloric acid, using thymolphthalein as indicator. From the titration obtained, corrected for a blank carried out on the reagents, calculate the percentage of formaldehyde from the following relationship.

Formaldehyde, % = ml. of 0·5 N HCl × 1·501/wt. of sample in grams.

SOME FURTHER APPLICATIONS OF THE METHOD DESCRIBED

The principle of these methods of examination of nylon and related polymers has been applied with success in the day-to-day work of a plastics laboratory. A sample of petrol tank material after removal of a thin surface coating was shown to consist of two types of nylon, one of which was soluble in alcohol and liberated formaldehyde on acidification. On hydrolysis it yielded 55 per cent. of adipic acid and 44 per cent. of hexamethylene diamine. This part of the sample was a formaldehyde modified 66 nylon. The other part was insoluble in alcohol, melted at 253° to 254° C. and yielded on hydrolysis 62 per cent. of adipic acid and 50·5 per cent. of hexamethylene diamine. This part was 66 nylon.

Synthetic brush monofilaments sent in for examination proved to consist of two parts.

(1) Fibres insoluble in *m*-cresol, 2¾ in. in length, which gave positive biuret and xanthoproteic reactions for protein. The nitrogen content of these fibres was 14·6 per cent.

(2) Fibres soluble in *m*-cresol, 4½ in. in length. On hydrolysis these yielded sebacic acid and hexamethylene diamine, and without doubt consisted of 610 nylon.

A sample from the head of a nylon spinning unit was shown to consist of nylon that had suffered considerable breakdown, as shown by the following figures:—

		Sublimate	Authentic 66 nylon
		%	%
1.	Moisture (loss after 4 hours at 100° C.)	1·24	—
2.	Ash	1·32	—
3.	Matter insoluble after HCl hydrolysis	7·2	nil
4.	Adipic acid recovered after HCl hydrolysis ..	49·5	63·0
5.	Residue recovered on evaporating with HCl the adipic acid-free hydrolysis product	82·8	81·0
6.	Chlorine in 5 calculated on original sample ..	31·8	37·2

SUMMARY

A method has been developed for the examination of nylon and related polymers. It is based on hydrolysis of the sample with hydrochloric acid solution followed by determination of the proportion and properties of the resulting organic acid and base hydrochloride. Examples are given of the application of the method to 66 nylon, 610 nylon, 6 nylon (caprolactam), 66 nylon modified with formaldehyde, and co-polymers.

A method has been devised for the determination of adipic and sebacic acids in mixtures of the acids as well as the isolation of pure specimens of the acids.

Applications of the methods in the day-to-day work of a Plastics Analytical Laboratory are described.

We should like to thank Mr. N. Payne for his assistance in the designing of the liquid/liquid extractor.

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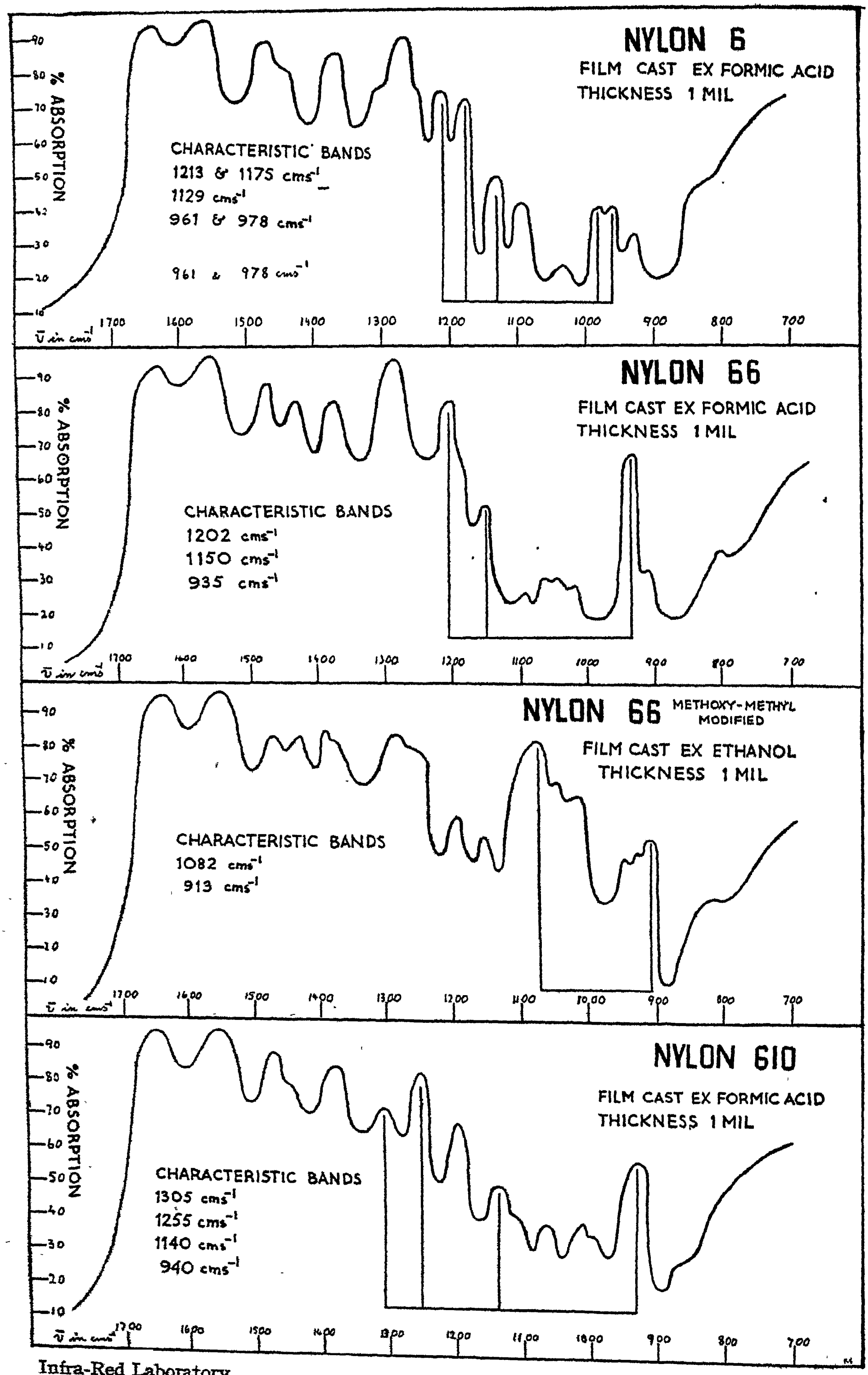

Infra-Red Laboratory
I.C.I. Plastics Division

Fig. 4 Infra-Red Spectra of Nylon Materials

Discussion

Mr. A. R. Munden asked if the authors could express an opinion on the value of physical methods, such as the use of infra-red spectra or X-ray diffraction, for the qualitative or quantitative analysis of nylon polymers.

Dr. R. E. Stuckey enquired what conditions were employed in the drying of the organic amine hydrochlorides in order to obtain quantitative results, and what methods were used to determine the total nitrogen content of various nylons.

Mr. R. Goulden asked if some particulars could be given about the determination of the melting-point of these polymers. Were the melting-points determined on polymer chips in air?

Mr. Haslam, in reply to Mr. Munden, pointed out that in all this work it was desirable to supplement chemical data by physical tests such as infra-red absorption spectra. Infra-red absorption spectra of four typical polymers prepared by Mr. H. A. Willis are shown in Fig. 4.

In reply to Dr. Stuckey it was pointed out that the organic amine hydrochlorides were dried at 100° C.; in the recovery of ϵ-amino caproic acid hydrochloride from solution in solvents it was always desirable to carry out a final evaporation with hydrochloric acid solution after removal of the solvent. The total nitrogen contents were determined by the macro-Kjeldahl procedure with selenium as catalyst.

Mr. Clasper, in reply to Mr. Goulden, indicated that the melting-points were determined in air without special precautions.

The Estimation of Acetylene in Air

By B. J. PURSER

In consequence of a requirement for a gas suitable for use in diffusion experiments, the estimation of acetylene in air came under review. The methods described in the literature proved unsuitable by reason of insufficient sensitivity, lack of means of storing the samples for any considerable time, and consequent restriction of value for the experiments envisaged. The methods also lacked flexibility in regard to the quantity sampled.

The principle adopted was to sample into an absorbent at a rate up to 2 litres per minute, and to determine the acetylene in the solution, or an aliquot part of it, at a later time. For the final determination of the acetylene, the copper acetylide reaction was considered the most promising, and attention was devoted to improving its sensitivity and method of application.

Choice of absorbent and reagent—

From existing information it was considered that acetone would be the best solvent for removing acetylene from air, but in view of its high volatility, other solvents, including solutions of copper salts, toluene, pyridine, alcohol and dibutyl phthalate, were tried. These proved unsatisfactory absorbents at the sampling rate required and acetone was finally adopted and used at a low temperature.

The reagent adopted for colour development was that used in the method of Coulson-Smith and Seyfang,[1] with the addition of gum ghatti instead of starch. Two ml. of this reagent for a test was found to be adequate. The acetone solution containing the acetylene was diluted with an equal volume of water before addition of the reagent. This amount of water was the minimum required to prevent the development of turbidity. It was found that the depth of colour produced was sensibly constant at 5 minutes after addition of the reagent, but that after 10 minutes a pronounced blue colour developed owing to oxidation of the copper salt.

Preparation of standard solution of acetylene—

In order to prepare a standard graph a solution of acetylene in acetone was made by passing a stream of acetylene from a Kipp's apparatus into acetone for a few seconds, and placing the resulting solution in a well-stoppered bottle. The strength of this stock solution was determined by treating a known volume with a solution of ammoniacal cuprous chloride. The resulting red precipitate, after washing, was analysed for copper. From the figure obtained, a suitable dilution of the stock solution with acetone was prepared for use in calibration.

METHOD

TRAPPING OF ACETYLENE—

For the absorption of the acetylene from an air stream in acetone a special design of bubbler was used, shown in Fig. 1. These bubblers are packed in solid carbon dioxide (Cardice); the number used in series depends upon the sampling rate and time of sampling. In general, three are used, containing 20 ml., 10 ml. and 10 ml. of acetone respectively. A maximum rate of 2 litres per minute for 15 minutes is permissible.

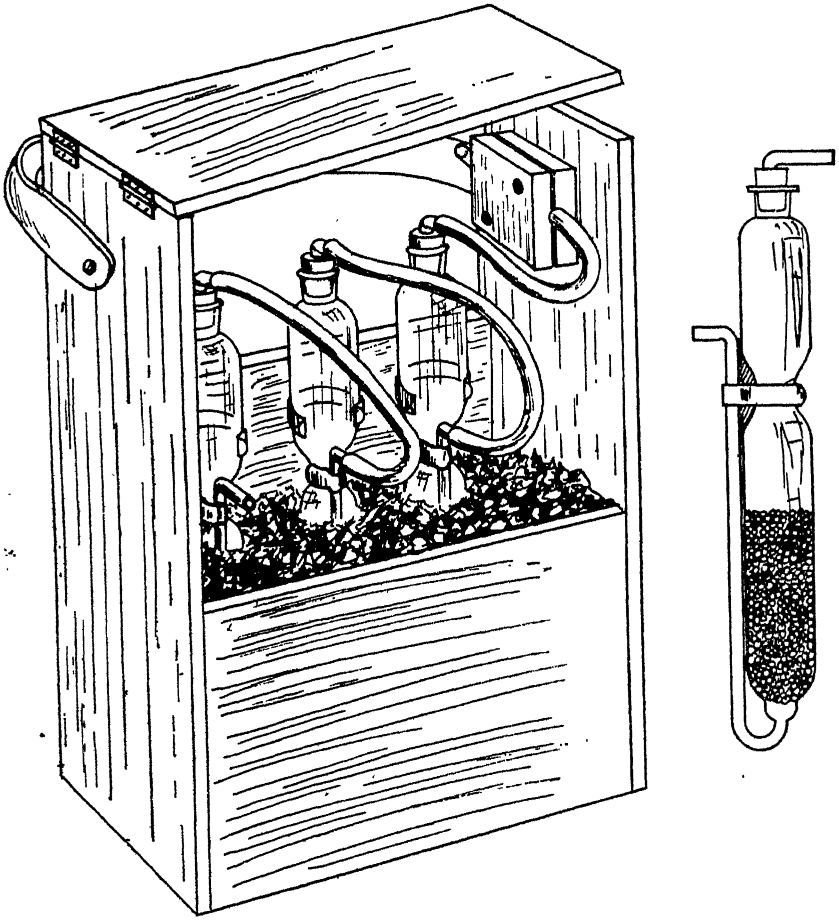

Fig. 1. Dimensions of the bubblers, height 10in., diameter 1¼in. Side tube diameter ¼in.

It was found that loss of acetylene from cold acetone during transference from one vessel to another or during storage up to 10 minutes in an open cylinder was not significant. The solutions may be stored in the bubblers surrounded by solid carbon dioxide for several hours without loss of acetylene.

No appreciable errors are caused by the coefficient of expansion of the acetone or measuring vessels over the temperature ranges used.

DETERMINATION OF THE ABSORBED ACETYLENE—

Reagent—To prepare the reagent, dissolve 0·8 g. of cuprous chloride A.R. in 20 ml. of water and 9 ml. of concentrated ammonia solution A.R. (sp.gr. 0·880). Then add 7 g. of hydroxylamine hydrochloride A.R. dissolved in 30 ml. warm water. After decolorisation, add 20 ml. of 1 per cent. gum ghatti solution and dilute the mixture to 100 ml. in a graduated flask.

The gum ghatti solution is prepared by suspending 1 g. of the gum, held in a muslin bag, in 100 ml. of water. This is allowed to stand for 24 hours. The gum solution is preferred to starch because it gives a clearer solution for estimation in a photo-electric colorimeter.

Into the reagent solution introduce a few strands of clean copper wire and allow 2 to 3 ml. of light petroleum to float on the surface. Under these conditions the reagent will keep for several days.

COLOUR PRODUCTION AND MEASUREMENT—

Do not remove the samples from the solid carbon dioxide until they are required for estimation. Empty the bubbler contents into a graduated flask, rinse with acetone and dilute the whole to a definite volume. Pipette an aliquot containing not more than 20 ml. of acetone into a 50-ml. graduated cylinder. If the aliquot is less than 20 ml., add acetone so that the final volume is 20 ml. Then add 20 ml. of water and mix. To this mixture add 2 ml. of the reagent and mix again. Allow the colour to develop for 5 minutes and read in the Spekker absorptiometer. Use No. 7 dark blue screens, and 4-cm. cells, with distilled water in the comparison cell.

Do not allow more than 10 minutes to elapse from the time the bubblers are emptied until the colour is developed. After the lapse of 1 minute it is possible to judge whether the aliquot has been suitably chosen. If not, repeat the estimation using a more suitable quantity.

CALIBRATION CURVES—

Values obtained from a standard acetylene solution were as follows.

Acetylene, mg. ..	0	0·01	0·024	0·034	0·049	0·058	0·073	0·097
Spekker reading ..	0·03	0·12	0·27	0·37	0·54	0·64	0·78	1·05

SUMMARY

A colorimetric method has been developed for the estimation of acetylene. The air sample is taken in a bubbler containing acetone cooled in solid carbon dioxide and subsequent analysis carried out with ammoniacal copper chloride in presence of gum ghatti, the red colour being measured in the Spekker absorptiometer and referred to a calibration curve.

The limit of sensitivity attainable is 1 part in 1·5 million parts of air by volume. The apparatus is portable and samples can be taken and stored prior to estimation if necessary. A maximum rate of 2 litres per minute for sampling is permissible.

With quantities of acetylene of 0·05 mg. and over the results are accurate to ±5 per cent.

The author wishes to express his thanks to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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New Method for the Separation of the Phosphate Ion in Elementary Qualitative Analysis

BY A. J. NUTTEN AND L. SABISTON

SEVERAL methods are available for the separation of the phosphate ion in elementary qualitative analysis, but the classical ferric chloride method is the one generally preferred. The retention of a method that is time-consuming and liable to yield faulty separations in unpractised hands is undoubtedly due to the fact that the alternative methods have some particular disadvantage which more than counterbalances any increased efficiency in the separation.

An ideal method should have the following characteristics—(1) The separation should be efficient. (2) As few steps as possible should be required in attaining complete separation. (3) The method should work satisfactorily in the hands of elementary students. (4) It should be capable of being superimposed on the conventional scheme without requiring any drastic alterations in the subsequent group separations. (5) The reagent should not be expensive. None of the alternative methods possesses all these features. In our opinion the most rapid and efficient separation proposed hitherto is that achieved by the use of salts of zirconium.

Two such procedures have been advanced: one using zirconium oxychloride[1] and the other a solution of zirconium nitrate[2] as the precipitating agent. The principle of the separation is the same with both; the reagent is added to the acid test solution until all the phosphate has been precipitated as zirconium phosphate and this is then separated by filtration. The filtrate is treated with ammonium chloride and ammonia solution whereby the iron group metals, together with excess of zirconium, are precipitated as hydroxides. This precipitate is examined for the iron group metals and the filtrate contains the metals of subsequent groups. The only objection to these two reagents is the high cost of zirconium salts. On the micro scale the method can be, and often is, applied without hesitation, but on the macro scale the cost is prohibitive.

It occurred to us that a less expensive reagent that could be used on the same principle might repay investigation, and accordingly the salts of titanium—whose phosphate is next to that of zirconium in order of insolubility—were examined.

Reagent—A solution of titanium hydroxide (ortho-titanic acid) in nitric acid was used as the reagent. To prepare it, excess of a solution of ammonia was added to a concentrated solution of titanyl ammonium sulphate* in distilled water, whereupon white titanium hydroxide, $Ti(OH)_4$, was precipitated. This was filtered at the pump, washed thoroughly with distilled water and immediately dissolved in concentrated nitric acid. Thorough washing of the precipitate is essential, to remove traces of sulphate, as this ion would interfere if metals of the alkaline earth group were present in the sample to be analysed.† It is also essential that the precipitate be dissolved immediately it has been washed, otherwise it becomes insoluble in nitric acid, owing probably to the formation of titanium dioxide.

The resulting solution of titanium hydroxide in nitric acid was diluted with distilled water to form a series of reagents of varying acid concentrations.

I. In a series of tests, 10 ml. of the reagent were added to 1-ml. portions of an aqueous solution of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$). The mixture was boiled for 1 to 2 minutes. When precipitation occurred, the precipitate was filtered, and the filtrate tested for phosphate by the zirconium nitrate method. In most cases where precipitation occurred with the titanium reagent, a white colloidal solution resulted on boiling, from which a precipitate formed either on cooling or on boiling a second time. Precipitation was accelerated by addition of a small amount (about 0·2 g.) of asbestos or paper pulp. The results are shown in Table I, in which the formation of a precipitate is indicated by a + sign and its absence by a − sign. In every instance where a precipitate was produced the filtrate from the precipitate was found to be free from phosphate.

TABLE I

ADDITION OF REAGENT IN NITRIC ACID SOLUTIONS TO PHOSPHATE IN AQUEOUS SOLUTIONS

Test solution of $Na_2HPO_4.12H_2O$, % in water	Nitric acid normality of reagent solution									
	17 *N*	10 *N*	8 *N*	7 *N*	6 *N*	5 *N*	4 *N*	3 *N*	2 *N*	1 *N*
4	−	−	−	+	+	+	+	+	+	+
3	−	−	−	+	+	+	+	+	+	+
2	−	−	−	+	+	+	+	+	+	+
1	−	−	−	−	−	−*	−*	+	+	+
0·5	−	−	−	−	−	−	−	+	+	+
0·25	−	−	−	−	−	−	−	−	−	−

* Cloudiness without precipitation.

It is seen that complete removal of phosphate is effected by the reagent in 1 *N* to 3 *N* nitric acid, with the phosphate at a concentration of not less than 0·5 per cent. of $Na_2HPO_4.12H_2O$.

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* Titanyl ammonium sulphate ($(NH_4)_2SO_4.TiOSO_4.H_2O$) is obtained by adding a concentrated solution of ammonium sulphate to a concentrated solution of titanium dioxide in concentrated sulphuric acid.

† More recently a solution of titanium tetrachloride, which is obtainable commercially, has been used to prepare the titanium hydroxide.

It may be mentioned at this point that the acid zirconium nitrate method gives a faint cloudiness, but no precipitate with a 0·25 per cent. phosphate solution. This indicates that the zirconium method is but little more effective than the titanium method.

IIA. In a further series of tests, 10 ml. of the reagent were added to 1-ml. portions of phosphate ($Na_2HPO_4.12H_2O$) solution in various concentrations of hydrochloric acid. The mixture was boiled as before, and the filtrate tested for phosphate by the zirconium nitrate method. In every instance where precipitation occurred the filtrate was free from phosphate.

TABLE IIA

ADDITION OF REAGENT IN NITRIC ACID SOLUTIONS TO PHOSPHATE IN HYDROCHLORIC ACID SOLUTIONS

Test solution of $Na_2HPO_4.12H_2O$	Nitric acid normality of reagent solution 17 *N*	10 *N*	7 *N*	5 *N*	3 *N*	1 *N*
4% in 5 *N* HCl	+	+	+	+	+	−
" " 2 *N* "	+	+	+	+	+	+
2% in 5 *N* HCl	+	+	−	−	−	−
" " 2 *N* "	+	+	+	+	+	+
1% in 5 *N* HCl	+	−	−	−	−	−
" " 2 *N* "	+	+	−	−	−	−

Table IIA shows that the phosphate becomes progressively more difficult to precipitate in solutions of hydrochloric acid as the concentration of this acid is increased from 2 *N* to 5 *N*. Thus the reagent in 1 *N* to 3 *N* nitric acid will not detect phosphate in a 1 per cent. solution if that solution is 2 *N* to 5 *N* with respect to hydrochloric acid; nor will it precipitate phosphate from a 2 per cent. solution in 5 *N* hydrochloric acid. In an attempt to obtain a more efficient separation of phosphate, precipitation was effected from 1 *N* hydrochloric acid solution.

IIB. Ten ml. of the reagent were added to 1-ml. portions of sodium phosphate ($Na_2HPO_4.12H_2O$) solution in 1 *N* hydrochloric acid. The mixture was boiled as before, and the filtrate tested for phosphate by the zirconium nitrate method. In every instance where precipitation occurred the filtrate was found to be free from phosphate.

TABLE IIB

ADDITION OF REAGENT IN NITRIC ACID SOLUTIONS TO PHOSPHATE IN 1 *N* HYDROCHLORIC ACID SOLUTION

Test solution of $Na_2HPO_4.12H_2O$ in 1 *N* HCl	Nitric acid normality of reagent solution									
	17 *N*	10 *N*	8 *N*	7 *N*	6 *N*	5 *N*	4 *N*	3 *N*	2 *N*	1 *N*
4%	+	+	+	+	+	+	+	+	+	+
3%	+	+	−	+	+	+	+	+	+	+
2%	+	−	−	−	−	−	−	+	+	+
1%	+	−	−	−	−	−	−	−	−	−
0·5%	−	−	−	−	−	−	−	−	−	−
0·25%	−	−	−	−	−	−	−	−	−	−

From Table IIB it is evident that in 1 *N* hydrochloric acid only strong solutions of phosphate (2 to 4 per cent. of $Na_2HPO_4.12H_2O$) give a precipitate with the reagent in 1 *N* to 3 *N* nitric acid, though it may be noted that the reagent in 17 *N* nitric acid completely removes phosphate from a 1 per cent. solution.

With the phosphate in 1 *N* hydrochloric acid the reagent is still not sufficiently effective in removing phosphate. Precipitation was therefore carried out in a phosphate solution made only slightly acid with hydrochloric acid.

III. Ten ml. of the reagent were added to 1-ml. portions of phosphate solutions containing, respectively, 4, 3, 2, 1 and 0·5 per cent. of $Na_2HPO_4.12H_2O$ in distilled water made slightly acid with hydrochloric acid. The reagent solutions used were 3 *N*, 2 *N* and 1 *N* in respect of nitric acid. The mixtures were boiled as before, and the filtrates tested for phosphate by the zirconium nitrate method. In every instance a precipitate was produced by the reagent and the filtrate was free from phosphate.

Hence complete removal of phosphate occurs with the reagent in 1 *N* to 3 *N* nitric acid, and with the phosphate solution made only slightly acid with hydrochloric acid, and at a phosphate concentration of not less than 0·5 per cent. of $Na_2HPO_4.12H_2O$.

The following procedure, based on these results, was then applied.

PROCEDURE

Boil the solution from the tin group until free from hydrogen sulphide, add a few drops of concentrated nitric acid, and boil to oxidise ferrous iron.

Test the solution for phosphate by evaporating a portion to dryness to remove hydrochloric acid, dissolving it in 8 *N* nitric acid, adding excess of a solution of ammonium molybdate and warming. A bright yellow precipitate indicates phosphate. If phosphate is present, remove it as follows.

To the filtrate from the group II separation, add ammonia solution till the liquid is neutral. Make slightly acid with hydrochloric acid, *i.e.*, until any precipitate formed by the addition of ammonia is just redissolved. Add a small amount of paper pulp and excess of the reagent (a solution of titanium hydroxide in 3 *N* nitric acid) and boil for 2 minutes. Cool and filter.

Residue

Titanium phosphate.
Reject

Filtrate

Nearly saturate with solid NH_4Cl and make ammoniacal, boil and filter.

Filtrate

Subsequent groups.

Residue

Contains Fe group and excess Ti. Continue the separation, using the following procedure:

Extract with boiling NaOH solution.

Residue

Add Na_2O_2 and water and digest. Filter.

Residue

Contains excess titanium; if brown, iron is present. Dissolve in HCl and test with KCNS.
A red colour ≡ **Fe.**

Filtrate

Acidify with acetic acid. Add lead acetate.
A yellow ppt. ≡ **Cr.**

Filtrate

Either acidify with HCl and add NH_3 solution.
Gelatinous ppt. ≡ **Al.**

or

Just acidify with dilute HCl add a few drops of ammonium acetate solution and 1 ml. of aurine tricarboxylic acid.
A bright red ppt. or colour ≡ **Al.**

IV. Finally, various phosphate mixtures containing most of the common cations were analysed; in all, the removal of phosphate was accomplished and the cations were detected by the usual methods. The reagent was used in 3 *N* nitric acid and the cation mixture made slightly acid with hydrochloric acid before addition of the reagent.

The cations used included Al, Fe, Cr, Co, Ni, Mn, Zn, Ca, Ba, Sr, Na, K and Mg.

The reagent itself is precipitated from dilute nitric acid solutions on boiling, but is stable in an acid concentration of 3 *N*.

The method has been used by students taking courses in qualitative analysis and has yielded satisfactory results in their hands. This method and the ferric chloride method were both taught to students, who were then allowed to use which method they preferred. All chose the new method.

SUMMARY

A new reagent is proposed for the separation of phosphate ion in qualitative analysis. The procedure for using it is rapid and gives excellent results in the hands of elementary students.

The reagent is a solution of titanium hydroxide in 3 *N* nitric acid, and the phosphate is removed immediately after the group II separation, from a solution that is just acid with hydrochloric acid. Addition of the reagent in excess, followed by boiling, gives a precipitate of titanium phosphate, which is removed by filtration. The filtrate is then tested in the usual manner for the group III metals, excess of titanium being precipitated along with the

metals of this group. A slightly modified procedure is necessitated for the analysis of group III; subsequent groups are analysed in the usual way.

Our thanks are due to Mr. R. Belcher, who suggested the investigation and for his interest throughout the work, to Mr. W. I. Stephen, who assisted during the development of the method, and to Dr. F. R. Williams of British Titan Products, Ltd., who supplied a sample of titanyl ammonium sulphate and suggested this salt as a suitable source for the preparation of the titanium reagent.

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DEPARTMENT OF CHEMISTRY
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The Volumetric Determination of Silica

BY H. N. WILSON

(Read at the Meeting of the Society on Wednesday, February 2nd, 1949)

THE determination of silica by measuring the yellow colour of silicomolybdic acid is now well known, but it is only recently that any attempts have been made to isolate the silica as an insoluble salt of the silicomolybdic acid. In 1947, a paper appeared by C. Duval[1] that described the semi-micro determination of silica by precipitation of the hexamethylenetetramine salt, which was washed and weighed. We investigated this method, but found it unsuitable for our purpose, which was to find a rapid accurate method for silica, suitable for routine use. Duval's method is too dependent on accurate control of pH; if the pH is too high the result is low through decomposition of the silicomolybdate complex, and if it is too low the hexamine is hydrolysed and again a low result is obtained. It may be that on a semi-micro scale the method would be more successful than we found it to be, working with rather larger quantities and hence more slowly.

The next important paper was by Brabson *et al.*,[2] who described the precipitation of the 8-hydroxyquinoline salt of silicomolybdic acid from a very acid solution. These authors had investigated the process in a very thorough way, and in our hands it proved entirely successful, fulfilling all their claims; but it is gravimetric, and we wished to have a volumetric method. It was thought that after solution of the precipitate in acid we could determine the 8-hydroxyquinoline in the usual way, but the precipitate is most extraordinarily insoluble; it can be dissolved in strong phosphoric acid, or hot concentrated hydrochloric acid, more easily in presence of oxalic acid, but on dilution either molybdic acid or some molybdate, probably that of 8-hydroxyquinoline, is precipitated; after trying every possible scheme we could think of, we reluctantly abandoned the attempt. It then occurred to us that we could perhaps dissolve the precipitate in standard caustic soda solution, and titrate back the excess. The precipitate is indeed readily soluble, but during its solution there is always produced a considerable amount of some deep blue product, which makes a titration impossible and must be the result of reduction of silicomolybdate.

This reduction was thought to be due to the hydroxyl group, so that if a similar insoluble salt without an OH group could be produced the volumetric method should be possible. The obvious choice was quinoline itself, so a solution similar in strength and acidity to Brabson's 8-hydroxyquinoline solution was prepared, and the original process repeated. An insoluble salt was produced, which was found to be soluble in caustic soda solution, though with slight production of molybdenum blue, and the resulting solution could be titrated with decinormal acid, thus proving that the scheme might be feasible. (The formation of molybdenum blue was due to presence of some impurity in the reagent; when the quinoline solution is prepared as described below, no reduction takes place.) There were many snags: the precipitate filtered badly and was quite difficult to dissolve in the dilute sodium hydroxide solution, apparently because once consolidated it was not readily wetted, but by systematically varying the conditions of precipitation we were able finally to work out a procedure that is rapid and accurate. The whole process takes an hour and a half, and for the kind of material in which we were primarily interested it compares favourably with the standard accurate method,[3] which cannot be completed in one working day.

REACTIONS INVOLVED—

To make clear the reasons for all the steps in the procedure, we preface its description with a brief account of its chemistry. After the silica is brought into solution as H_4SiO_4, the reactions are analogous to those of the volumetric method for H_3PO_4.

Solution of the sample—In the usual methods for determining silica the end is to obtain more or less pure silica as insoluble SiO_2; in this method it must be retained in solution as ortho-silicic acid until it is combined with the molybdic acid. The most generally useful method of attack is to fuse with sodium hydroxide. The reaction is violent, and unless care is taken silica is liable to be lost.

The caustic fusion is extracted with water, and the extract poured into excess of hydrochloric acid. In this way the whole of the silica is retained in solution.

$$\mathrm{Na_2SiO_3 + H_2O + 2HCl = H_4SiO_4 + 2NaCl} \qquad (1)$$

To ensure that none of the unstable silicic acid has become dehydrated and polymerised, the acid solution is cooled and again made alkaline with sodium hydroxide, neutralised to a pH of about 2, and such an excess of hydrochloric acid added that after addition of ammonium molybdate solution the pH will be about 1·5, the optimum value for the rapid formation of silicomolybdic acid.

$$\mathrm{H_4SiO_4 + 12(NH_4)_2.MoO_4 + 24HCl = H_4[SiO_4.12MoO_3] + 24NH_4Cl + 12H_2O} \qquad (2)$$

This reaction is quantitative if a moderate excess of ammonium molybdate is present. The silicomolybdic acid is very sensitive to the presence of OH ions, but very stable in strongly acid solutions, though its formation is slow except at about the correct pH.

After the complex is formed the solution is made strongly acid before the quinoline reagent is added. Unless this is done the precipitate will be contaminated with quinoline molybdate (soluble in strongly acid solution, insoluble in weakly acid solution). This salt is often precipitated during filtration, when the acid filtrate is diluted by the washings. On addition of the quinoline hydrochloride solution the insoluble salt is precipitated.

$$\mathrm{4C_9H_7NHCl + H_4[SiO_4.12MoO_3] = (C_9H_7N)_4H_4[SiO_4.12MoO_3] + 4HCl} \qquad (3)$$

(It is interesting to note that the corresponding pyridine salt is too soluble and that neither naphthylamine nor aniline gives insoluble salts.)

On solution of the precipitate in caustic soda solution the quinoline is set free, but is too weak a base to interfere and the silicic acid similarly is too weak an acid, so that in effect only the molybdic anhydride functions as an acid.

$$\mathrm{(C_9H_7N)_4H_4[SiO_4.12MoO_3] + 24NaOH = 4C_9H_7N + SiO_2 + 2H_2O + 12Na_2MoO_4} \qquad (4)$$

Thus in this reaction the equivalent weight of silica (SiO_2) is one twenty-fourth of its molecular weight—60·06/24 = 2·5025. A convincing demonstration of the quantitative nature of the reactions is given by the equivalent weight ascertained in a very large number of experiments, *viz.*, 2·513, the slight difference being accounted for by very small losses during the washing of the precipitate with water.

METHOD

REAGENTS—

Quinoline solution, 2 per cent.—Purify the quinoline (technical pure) by fractional distillation. Reject any fore-runnings below 230° C. and collect the fraction between that temperature and 240° C. (pure quinoline boils at 237·7° C.). Add 20 ml. of quinoline to about 800 ml. of hot water, acidified with 25 ml. of concentrated hydrochloric acid, stirring constantly. Cool the solution to room temperature and add some paper pulp, stirring vigorously. Allow to settle and filter with suction through a pulp pad on a small Buchner funnel. Suck the filter dry, but do not wash. This process is to remove traces of oily matter from the solution. Dilute the filtrate to 1 litre with distilled water.

Ammonium molybdate solution, 10 per cent.—Dissolve 50 g. of pure ammonium molybdate in 500 ml. of water; allow to stand 24 hours and filter. This solution may take up silica from the glass bottle, and should be used within a week.

Thymol blue indicator solution—Dissolve 0·4 g. in 200 ml. of ethyl alcohol, add 8·6 ml. of 0·1 *N* sodium hydroxide and dilute to 1 litre with water.

Cresol red - thymol blue indicator solution—Grind 0·1 g. of cresol red with 5·3 ml. of 0·1 *N* sodium hydroxide until dissolved and dilute to 100 ml. with water. Dissolve 0·1 g. of thymol blue in 20 ml. of ethyl alcohol, add 2·1 ml. of 0·1 *N* sodium hydroxide solution and dilute to 100 ml. with water. Mix the two solutions.

Acetic acid—33 ml. of glacial acetic acid diluted to 100 ml. with water.

Hydrochloric acid, concentrated; diluted $(1 + 1)$; diluted $(1 + 9)$; 0·5 *N* standard solution.

1 *N sodium hydroxide solution*, free from carbon dioxide.

PROCEDURE—

The method as set out below is suitable for samples containing a maximum of 12 per cent. of SiO_2. If more than this is present the extract from the fusion must be made up to a convenient bulk, say 250 ml., and a suitable aliquot taken for analysis. Or alternatively, a smaller sample than 500 mg. may be weighed out, but in no case must more than 60 mg. of silica be present in the portion finally taken for analysis.

Fusion—If any organic matter, carbon, coke dust, etc., be present, ignite 0·5000 g. of the sample (ground in an agate mortar) at 950° to 1000° C. in a muffle furnace until the carbonaceous matter is burnt off. Fifteen minutes usually suffice. Allow the sample to cool.

Weigh 7 g. of AnalaR sodium hydroxide into a nickel crucible (5 cm. diameter) and fuse over a bunsen burner until the water is driven off and a quiet melt is obtained. Allow to cool.

Carefully brush the 0·500 g. sample (after ignition if necessary) into the nickel crucible, and gently fuse over a medium bunsen flame for 3 minutes, finishing at a dull red heat.

(Some samples of very refractory materials may take longer, but 3 minutes is usually long enough. Samples rich in silica ($>$20 per cent.) must be treated very carefully at this point; the reaction is violent, and portions of the sample may be lost through spraying. For very reactive samples it is best to add 4 or 5 ml. of alcohol and a drop of water to the crucible, slowly evaporate off the alcohol, cover the crucible with a lid, and put it on a really hot plate until the caustic slowly fuses, finally removing the lid and finishing over a bunsen burner. In either case attack on the nickel crucible is very small. Apart from the saving in time there is no reason why sodium carbonate fusion should not be used: it would then be preferable to extract with dilute NaOH solution.)

Solution—Allow the crucible to cool until hand-warm and then place it in an upright position in a tall-form 500-ml. beaker and cover with a watch-glass. Raise the cover slightly, and from the blowing tube of a wash bottle fill the crucible with water *at the boiling-point* and at once replace the cover. The crucible should be at such a temperature that the heat of solution keeps the water boiling until all is dissolved, but not so hot that liquid is thrown out of the beaker.

When the vigorous reaction is over, wash down the cover glass and the sides of the beaker. Remove the crucible with a pair of tongs, carefully wash it inside and out with hot water. No undissolved melt should remain, but, as a precaution, rinse it with a few ml. of warm dilute hydrochloric acid. Do not add the acid washings to the alkaline extract but collect them in a 500-ml. "iodine flask" which should be marked at 170 ml.

Measure 20 ml. of concentrated hydrochloric acid into the "iodine flask" and pour the alkaline extract into the acid, swirling constantly during the addition. Complete the transfer with a jet of hot water. Heat the flask on a hot-plate until the solution clears, but do not boil. Remove from the hot-plate, cool rapidly to room temperature and dilute to 170 ml. with distilled water. Add 3 g. of solid sodium hydroxide (most conveniently as pellets) and swirl the flask until it is dissolved. Add 8 drops of thymol blue indicator, then concentrated hydrochloric acid drop by drop from a dropping pipette, swirling constantly, until the indicator changes from blue, through yellow, just to red. The solution should clear again in a few minutes.

Precipitation—Add 8 ml. of diluted hydrochloric acid $(1 + 9)$, 5 ml. of diluted acetic acid $(1 + 2)$ and then 30 ml. of 10 per cent. molybdate solution, mixing well by swirling during the addition of the reagents, and continuing to swirl vigorously for 1 minute after the molybdate solution has been added. Immerse the flask in a boiling water-bath, and raise the temperature of the contents to 80° to 90° C. during 10 to 12 minutes.

Remove from the bath, add 40 ml. of diluted hydrochloric acid (1 + 1) and immediately precipitate the silica by running in 65 ml. of quinoline solution from a burette, swirling the flask constantly during the addition. The burette should have a much wider jet than usual, but the solution must not be poured in from a measure.

Return the flask to the hot water-bath and raise the temperature again to 80° to 90° C. for a further 5 minutes, swirling occasionally. Cool rapidly to 15° C. or less, and allow the precipitate to settle.

Filtration—Fit a 3-in. funnel with a porcelain filter cone and prepare a good paper pulp pad, well tamped down, and filter with suction. Decant the liquor as cleanly as possible from the precipitate and suck dry before commencing to wash. Wash twice by decantation with about 25 to 30 ml. of cold water and transfer the precipitate to the pad. Wash the precipitate thoroughly with six 30-ml. portions of *cold* ($<15^\circ$ C.) water, sucking dry after each wash.

Titration—Remove the precipitate and pad from the funnel (*e.g.*, by blowing them out on to a small paper filter) and transfer to the original flask. Dissolve in 30·0 ml. of *N* sodium hydroxide solution. This is best done by running the sodium hydroxide solution down the sides of the funnel into the flask, thus removing any small traces of precipitate that may adhere to the glass. Wash down the funnel thoroughly with distilled water, using no more than necessary. Close the flask with a stopper, and shake vigorously until all the precipitate has dissolved, being careful that no small yellow specks remain among the paper pulp.

Add a few drops of cresol red - thymol blue indicator, and titrate back with 0·5 *N* hydrochloric acid until the indicator, at first blue, just changes through rose colour to yellow. Calculate the volume of 1 *N* sodium hydroxide used up by the molybdic acid.

Carry out a "blank" determination on all the reagents; most of the "blank," which usually amounts to about 0·6 ml., is due to the sodium hydroxide.

Deduct the "blank" 1 *N* sodium hydroxide from the main titration and calculate the silica from the factor: 1 ml. of 1 *N* sodium hydroxide ≡ 0·002513 g. of SiO_2.

The whole process (excluding the determination of the blank) can be carried out in 1½ hours if the silica content is less than 20 per cent. and special precautions need not be taken with the caustic fusion.

REMARKS—

This method of analysis was devised in response to a request for a rapid method for determining silica for process control work, but results were so good that the investigation was extended to cover a few common siliceous materials. We have not gone thoroughly into the problem of interfering elements, but it is already clear that the method is fairly widely applicable. Phosphates interfere, but the usual constituents of, *e.g.*, fireclay or cement, do not. Although Portland cement dissolves in dilute acid, if the silicomolybdate process is to be applied the cement must be fused with alkali as described above; the silica dissolved in dilute acid does not react completely.

REPRODUCIBILITY AND ACCURACY OF RESULTS—

The table shows a collection of representative results, compared with those by the usual methods. As we are largely interested in materials containing anhydrite many of the results are on this class of material.

SYSTEMATIC ERROR AND STANDARD DEVIATION—

As can be seen from the table there is no systematic error. The standard deviation, calculated from all the groups of replicate analysis, is 0·048. (This includes the group of analyses of sample 21: these analyses were the work of one analyst during a period of about two months, each figure being obtained independently. It is therefore permissible to include so large a group on one sample by one analyst in the calculation of the standard deviation.) This method for silica is thus more precise than the classical method of Hillebrand, of which the standard deviation was 0·083 for a similar class of material.

SUMMARY

A new method is described for the determination of silica. The silica is converted to silicomolybdic acid and precipitated as quinoline silicomolybdate from a strongly acid solution.

TABLE

COMPARISON BETWEEN GRAVIMETRIC AND THE VOLUMETRIC METHOD

Nature of sample	SiO$_2$ present %	SiO$_2$ found volumetrically		Analyst
		Average %	Individual results %	
Miscellaneous samples.				
Anhydrite ("pure")	0·20 (*a*)			H.N.R.
Anhydrite	3·48 (*a*)	3·50	3·53	S.L.
			3·47	G.W.L.
"	3·88 (*a*)	3·77	3·80	S.L.
			3·74	G.W.L.
Mixture of (1) with pure quartz	5·17 (*c*)	5·15	5·15	H.N.R.
" " " " "	20·10 (*c*)	19·93	19·93	"
Cement (*d*)	22·0 (*b*)	21·89	21·91	"
			21·86	
" (*d*)	21·5 (*b*)	21·36	21·41	"
			21·31	
" (*d*)	20·7 (*b*)	20·91	20·91	"
			20·91	
Cement clinker	21·44 (*a*)	21·46	21·46	F.V.
Fireclay	50·00 (*a*)	50·26	50·26	H.N.R.
Refractory brick	62·07 (*a*)	62·13	62·19	"
			62·07	
Limestone (*d*)	7·0 (*a*)	7·14	7·14	J.H.
			7·14	
Silicon iron (*d*)	15·0 (*a*) as Si	15·48 as Si	15·66 15·36 } as Si	H.N.R.
Impure anhydrite samples.				
Impure anhydrite (*e*)	10·06 (*a*)	10·04	10·07 10·03	"
			10·03 10·05	
			10·12 10·03	
			10·05 9·98	
			9·95 10·03	
			10·03 10·07	
Mixture (21) + (1) (*d*)	8·16 (*c*)	8·15	8·17	"
			8·17	
			8·10	
" (21) + pure quartz (*d*)	12·31 (*c*)	12·13	12·12	"
			12·14	
" " (*d*)	13·15 (*c*)	13·06	13·11	"
			13·01	
" (21) + (1) (*d*)	8·81 (*c*)	8·76	8·72	S.H.
			8·75	
			8·82	
		8·79	8·75	T.D.R.
			8·84	
Contaminated anhydrite (*d*)	9·37 (*a*)	9·49	9·42	F.V.
			9·50	
			9·55	
			9·52	
			9·47	
" "	10·12 (*a*)	9·92	9·88	B.P.
			9·90	
			9·93	
			9·95	
			9·95	F.V.
" "	8·99 (*a*)	8·97	8·90	B.P.
			9·00	
			9·02	
" " (*d*)	9·07 (*a*)	9·32	9·37	F.V.
			9·27	
" " (*d*)	13·58(*a*)	13·66	13·64	"
			13·69	

NOTES TO TABLE—

(*a*) Silica determined in duplicate by "standard"[3] method of rock analysis: the standard deviation of a silica determination has been determined as 0·083 per cent.

(*b*) Silica determined by the routine method of cement analysis.

(*c*) Mixtures made from the stated constituents.

(*d*) The composition of the sample was unknown to the analyst carrying out the determination.

(*e*) The method was developed using this sample. The quoted value, 10·06 per cent., for the SiO_2 present is a mean of closely agreeing duplicates, 10·03 and 10·10 per cent., by two analysts. Some of the 12 volumetric results cited were obtained before the method was standardised into its present form.

Of the eight analysts taking part, none except H.N.R. had any previous experience with the method.

The quinoline silicomolybdate is dissolved in excess of standard sodium hydroxide solution, and the excess titrated back with standard acid. The method is rapid and has a smaller standard deviation than the orthodox method.

The author wishes to thank Mr. H. N. Redman for his share in the work described above, as the whole of the experimental work during the development of the method and the majority of the analyses quoted were carried out by him.

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RESEARCH DEPARTMENT
PHYSICAL CHEMISTRY GROUP
I.C.I., BILLINGHAM DIVISION

DISCUSSION

The PRESIDENT congratulated the author on an interesting paper and enquired as to the possibility of distinguishing between free and combined silica.

Mr. A. L. BACHARACH asked if the author had any information as to whether his method was suitable for determining small amounts of free or combined silicon in biological material.

Mr. R. F. MILTON said that he was particularly interested in this paper because he had once had to investigate the possibility of separating small amounts of silica, arsenate and phosphate, using molybdate combined with the same type of base. One got almost quantitative precipitation of the three, but their separation was by no means simple and in biological work the large amount of phosphorus present was an added difficulty. He enquired whether the author had had any experience in this direction or of the effect of an excess of base.

Dr. K. D. LUKE asked if the method was suitable for assaying quartz sand containing 99 per cent. or more of silica, and was there any particular reason for using sodium hydroxide rather than the carbonate in the fusion?

Mr. C. H. MANLEY asked if a mixture of sodium and potassium carbonates had been tried for the fusion.

Mr. F. L. OKELL enquired as to the type of crucible used for the fusion.

Mr. WILSON thanked the President for his remarks, and in reply to his query said that silicon, however combined, would be converted to ortho-silicic acid, and so various compounds could not be distinguished. In reply to Mr. Bacharach, he said that he thought most biological material would contain so much phosphorus as to render the method inapplicable. He had not investigated the effect of phosphates or arsenates; it was obvious that they would interfere; with phosphate he would expect the reaction to be stoicheiometric, as was indicated in Brabson's paper. Mr. Milton had asked about the effect of excess of base, but so long as excess was present as hydrochloride, it did not seem to be critical how large the excess was; correct control of acidity is, however, important. In reply to Dr. Luke, he said the only reason for the use of sodium hydroxide was speed, as both fusion and solution of the melt were very rapid. The reaction was very vigorous, and he thought that caustic fusion would not be convenient for quartz sand and the like; he had not tried fusion mixture. The crucible used was the ordinary nickel crucible, 5 cm. in diameter; attack on the nickel was small. Silver capsules or crucibles were very satisfactory, and iron crucibles could also be used. For fusion with sodium carbonate or fusion mixture one would use platinum.

A Method of Determining the Tin Content of Tungsten High-Speed Tool Steel

BY B. BAGSHAWE AND E. DYKE

(Read at the Meeting of the Society, on Wednesday, November 3rd, 1948)

THIS investigation was undertaken to meet a demand for information on the incidence of tin contamination in high-speed tungsten steel. High tungsten steels have always been liable to contamination with tin, cassiterite (tinstone) being a native impurity in many tungsten ore deposits, and the extent of the contamination from this and other sources appears to have assumed more serious proportions under war and post-war conditions.

The most common method of determining tin, consists of reducing it from the quadrivalent to the bivalent condition with a metallic reductant and measuring its re-oxidation to the quadrivalent state with standard iodine or iodate solution. The reducing metal may be antimony, aluminium, iron, nickel, lead or combinations of these.

In the present investigation, reduction has been made with metallic aluminium in presence of a dissolved antimony salt.[1] The main feature of this variant of the more usual form of antimony metal reduction is that metallic antimony is chemically precipitated in the solution in a state of uniform and reproducible fineness, and hence of uniform chemical reactivity, whereas reductions made with added antimony metal are liable to inconsistencies associated with fluctuations in the fineness and reducing activity of the added metal.[2] This direct reduction method is restricted to plain and low alloy steels; it can be applied directly to a simple solution without any separations, but is not directly applicable to solutions of high-speed and other complex alloy steels.

The object of the present work has been to find suitable means of extending its application to tungsten high-speed tool steels, a class of products that has provided a difficult problem to the analyst.

The main problem with high-speed steels is not the reduction and titration of the tin but rather one of quantitatively isolating the tin from interfering constituents and obtaining it in a solution suitable for the reduction technique.

Tungsten interferes seriously in the reduction and titration of tin solutions, hence all tungsten must be removed and provision made for recovering occluded tin which tungstic acid precipitates are prone to carry. The residual solution contains high concentrations of chromium, vanadium, cobalt, etc. which, even if chemically non-interfering, seriously impair the sensitivity of end-point detection owing to the strong colour of their salt solutions.

Preliminary experiments showed that adsorptive losses of stannic oxide in the tungstic acid hydrolysis product from these high-tungsten steels (containing 14 to 21 per cent. of tungsten) invariably occur, but the loss is variable and uncertain and may account for up to 10 per cent. of the tin present. Provision for its recovery can be made by sulphide separations of the tin from the soluble potassium bisulphate fusion extract of the ignited tungstic acid, in tartaric acid medium, but the manipulation is laborious and liable to error.

The alternative procedure to initial tungstic acid hydrolysis, in which the tungsten is complexed in the steel solution as citrate or tartrate,[3] also requires very careful manipulation and depends on a sulphide separation of the tin from tungsten-rich solutions, in which relatively large amounts of ferric iron must also be reduced by the precipitating reagent.

SEPARATION OF TUNGSTEN AS CARBIDE—

It occurred to us that it might be possible to dissolve the steel in a non-oxidising acid and thus remove tungsten at the outset, as unattacked stable carbide and tungstide. A carbide residue of this type is much denser and more manageable than its corresponding tungstic acid decomposition product and it seemed likely that the non-reactive carbide would filter cleanly without co-precipitation of tin. It might, therefore, be possible to effect a major simplification of procedure, by removing and discarding the tungsten as carbide. Further, the tin-bearing solution, being in a non-oxidised condition, would be obtained directly in a more suitable form for the tin sulphide precipitation.

A detailed examination of the residues from a large number of high-speed steels confirmed the simplicity and efficacy of this method of removing tungsten.

High-speed steels dissolve readily in hydrochloric acid and if excessive evaporation, or localised drying of salts on the beaker, or other circumstances favourable to partial oxidation are avoided, it is possible to obtain complete solution of tin, together with iron and the metallic portion of the sample, leaving a dense unattacked, non-adsorptive residue of carbide and tungstide which contains all, or all but traces of, the tungsten.

The method adopted to prove the absence of tin contamination in these separated residues was as follows.

The residues, together with the filter, were decomposed with 25 ml. of nitric acid (sp.gr. 1·42) and 10 ml. of sulphuric acid (sp.gr. 1·84) and fumed to expel nitric acid. The sulphates were taken up with 50 ml. water, 15 ml. of 50 per cent. tartaric acid solution, neutralised with a 1 + 1 solution of aqueous ammonia to dissolve tungsten and then acidified with 2 ml. of sulphuric acid (sp.gr. 1·84). This solution was heated to boiling and saturated with hydrogen sulphide and tin determined on a solution of the filtered precipitates by the usual reduction and titration technique. In no case was a titration of more than 0·2 ml. of 0·01 *N* iodate obtained. This value is only comparable with a blank titration and it was clear, therefore, that removal of tungsten as carbide was practicable.

The validity of this conclusion was further confirmed, at a subsequent stage, when improvements in the technique of separating trace amounts of tin as sulphide were adopted.

THE SULPHIDE SEPARATION—

The removal of tungsten, and the reduction and titration of tin having been dealt with, it only remained to test the validity of the tin sulphide separation itself for the amounts of tin being handled and the steps necessary for isolating the tin from co-precipitated sulphides. A careful examination of the sulphide precipitation from pure solutions of known tin content showed that precipitation of such small amounts of tin as 1 to 4 mg. (the usual working range) was apt to be erratic and usually tended to be incomplete. Thus, in two particularly bad instances, we recovered 0·0009 g. and 0·0012 g. of tin, respectively, from two tin sulphide filtrates starting from an initial quantity of 0·005 g. of tin. We considered that the erratic and low recovery was probably due to absence of nuclei to promote precipitation and that if this was so, the addition of copper or molybdenum would produce a precipitate that would promote and "carry" the tin sulphide precipitate.

Tests were made on pure tin solutions, with and without addition of copper and molybdenum salts, and the results (Table I) indicated the need for a "carrier" sulphide.

TABLE I

Tin present equivalent to 8·1 ml. of 0·01 *N* KIO_3

	Tin recovered, in terms of ml. of 0·01 *N* KIO_3
(*a*) No additions	5·10; 7·45
(*b*) + 0·05 g. of copper	8·40; 8·50
(*c*) + 0·05 g. of molybdenum	8·10; 8·05

Copper causes a rather high result owing to its partial retention throughout the subsequent operations. This error would probably disappear if the amount of copper was reduced to 0·01 g., which would still probably be sufficient to act as a sulphide carrier. In view of the satisfactory behaviour of molybdenum, however, it was considered unnecessary to proceed further with copper.

On the basis of the foregoing observations the following procedure was adopted.

METHOD

SPECIAL SOLUTIONS REQUIRED—

Ferric chloride solution—Dissolve 0·5 g. of pure tin-free iron with 20 ml. of hydrochloric acid (sp.gr. 1·16), oxidise with the minimum amount of nitric acid (sp.gr. 1·42), boil to expel nitrous fumes and dilute to 100 ml.

Antimony chloride solution—Treat 0·5 g. of powdered antimony with 5 ml. of sulphuric acid (sp.gr. 1·84) and heat to fuming and fume for 5 minutes. Cool, dissolve in a mixture of 20 ml. of hydrochloric acid (sp.gr. 1·16) and 50 ml. of water, boil for 5 minutes, cool and dilute to 100 ml.

Standard potassium iodate solution, 0·01 *N*.

Note. It is important that all solutions used after the reduction stage, *i.e.*, bicarbonate, iodide, starch and iodate solutions, should be prepared with freshly boiled distilled water to ensure maximum freedom from dissolved oxygen.

PROCEDURE—

To 5 g. of sample contained in a 600-ml. beaker add 60 ml. of hydrochloric acid (sp.gr. 1·16) and digest until the metal is completely disintegrated and only the insoluble carbide residue remains. Concentration of the acid by evaporation should be avoided; it is sufficient to allow the mixture merely to simmer throughout the solution process. When solvent action ceases, dilute immediately with 50 ml. of hot water, heat just to boiling, remove from the source of heat, and set aside for a few minutes to allow the carbide residue to settle. Filter through a tight pulp pad, as far as possible by decantation, wash with hot diluted hydrochloric acid (1 in 20) and discard the filtered residue.

Add 5 ml. of bromine water to the filtrate and boil for a few minutes. Neutralise the free acid by careful additions of diluted aqueous ammonia (1 + 1), until a permanent precipitate is just obtained. Just discharge this precipitate with a little diluted hydrochloric acid (1 in 20), and then add 5 ml. of hydrochloric acid (sp.gr. 1·16) in excess. Dilute the solution to approximately 450 ml., add 2 ml. of a 1 per cent. w/v solution of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, heat to boiling and saturate the solution with hydrogen sulphide gas for 1 hour. Allow to stand, preferably overnight, and then filter the solution through a pulp pad and wash with diluted sulphuric acid (1 in 100) that has previously been saturated with hydrogen sulphide.

Transfer the sulphide precipitate together with the filter to the original beaker, pour over it 25 ml. of nitric acid (sp.gr. 1·42), followed by 10 ml. of sulphuric acid (sp.gr. 1·84). Evaporate to dense fuming to ensure complete destruction of organic matter, adding more nitric acid if necessary, until there are no further indications of charring and a clear syrupy concentrate is obtained.

Extract the fumed concentrate by warming with 100 ml. of water and 15 ml. of hydrochloric acid (sp.gr. 1·16). A few milligrams of tungstic acid may separate at this point and if this occurs filter through a pulp pad and wash with diluted hydrochloric acid (1 in 20). To the solution add 5 ml. of ferric chloride solution (see p. 250) and then add diluted aqueous ammonia (1 + 1) to the boiling liquid until alkaline. Filter the precipitated hydroxides of iron and tin through a Whatman No. 541 paper and wash with hot water. Dissolve the precipitate from the paper with 140 ml. of hot diluted hydrochloric acid (1 + 1), collecting the extract in a 400-ml. Taylor-pattern flask, and finally wash the paper several times with hot diluted hydrochloric acid (1 in 20), collecting these washings also in the flask containing the main extract.

Add 2 ml. of antimony chloride solution (see p. 250) and dilute with water to a total volume of approximately 200 ml. Heat nearly to boiling and then add carefully, a small amount at a time, 0·5 g. of tin-free aluminium, in the form of drillings or thin foil. Before the last portion of aluminium has completely dissolved, stopper the flask with a bung carrying a bent delivery tube which overhangs the edge of the hot plate. Heat to boiling and maintain in this condition for 30 minutes, throughout which time the condensate from the tube can be allowed to drip into a beaker for discard. At the end of the 30-minute period, immerse the end of the delivery tube in 200 ml. of sodium bicarbonate solution (10 g. per 100 ml.) and then cool the flask rapidly in running water; the sodium bicarbonate is sucked into the flask throughout this cooling period and maintains an atmosphere of carbon dioxide over the reduced tin solution. Alternatively the use of sodium bicarbonate may be avoided by passing a steady current of carbon dioxide through the solution during cooling.

Remove the bung, add 10 ml. of potassium iodide solution (10 g. per 100 ml.) and a few ml. of starch solution and titrate without further delay with 0·01 *N* potassium iodate solution to the first permanent appearance of the starch blue colour.

1 Ml. of 0·01 *N* potassium iodate ≡ 0·0005935 g. of tin, equivalent to 0·0119 per cent. of tin on a 5-g. sample.

A blank determination on the reagents should be conducted through all stages of the process, using 5 g. of pure tin-free iron as base.

The tin value of the 0·01 *N* potassium iodate may be checked by carrying out an additional test on 5 g. of pure tin-free iron and adding a known amount of standard tin solution just before the reduction with aluminium.

RESULTS

The method was tested on high-speed steels and its validity confirmed for tin contents up to 0·2 per cent. by comparative tests in which certain samples were treated with different amounts of standard tin solution. The results, which show consistently satisfactory recovery, are shown in Table II.

TABLE II

Steel (18% W, 4% Cr, 1·5% V)			Titration, ml. of 0·01 N KIO_3	Tin found, %
A	(*a*)	No additional tin	3·3	0·039
	(*b*)	" " "	3·4	0·040
	(*c*)	" " "	3·3	0·039
	(*d*)	+ 0·10% added tin	11·3	0·134
	(*e*)	+ " " "	11·7	0·139
	(*f*)	+ " " "	11·55	0·137
	(*a*)	No additional tin	2·7	0·031
	(*b*)	+ 0·10% added tin	10·85	0·130
	(*c*)	+ 0·20% " "	18·55	0·223
	(*a*)	No additional tin	2·4	0·028
	(*b*)	+ 0·10% added tin	10·5	0·126

SUMMARY

A method for the determination of tin in high-speed steel is described, which has for its essential features a separation of tungsten as the carbide from non-oxidising acid media, the removal of tin as the sulphide and its subsequent reduction to the bivalent state with metallic aluminium in presence of an antimony salt and titration of the reduced tin with standard iodate solution.

Previously used procedures, in addition to being cumbersome and laborious, are subject to inaccuracy due to adsorptive losses of tin in tungstic acid and inconsistencies in the quantitative separation of small amounts of tin as the sulphide.

It is shown that by initially removing the tungsten as unattacked carbide, thus avoiding its oxidation to, and separation as, tungstic acid, adsorption losses are avoided. The erratic behaviour of small amounts of tin to precipitation as the sulphide appears to be due to absence of sufficient precipitate-forming nuclei, and can be remedied by the simple expedient of adding a small concentration of molybdenum salt, the precipitated sulphide acting as "carrier" and ensuring quantitative co-precipitation of tin.

The authors are indebted to the Directors of Thos. Firth & John Brown, Ltd., and in particular to Dr. C. Sykes, F.R.S., Brown - Firth Research Laboratories, for permission to publish this paper.

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2. Evans, B. S., and Higgs, D. J., *Analyst*, 1944, **69**, 291.
3. Lundell Hoffman, and Bright, *Chemical Analysis of Iron and Steel*, p. 385.

THE BROWN-FIRTH LABORATORIES
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DISCUSSION

Mr. F. L. OKELL said that the method of separating tungsten as tungstide marked an advance in the analytical chemistry of alloy steels. He predicted that it would find many applications in the analysis of ferrous alloys in addition to that for which it had been devised by the authors.

The B.I.S.R.A. method for the volumetric determination of tin, which had been accepted and adopted by the authors, and which depended upon the reduction of stannic chloride by means of metallic aluminium in a solution containing antimony had been shown by Evans and Higgs in two recent papers (*Analyst*, 1944, **69**, 201 and 291) to be unreliable and a study of their work would suggest that an accurate tin assay was probably due to a balance of errors. It was within his own experience, using the procedure described by Okell and Lumsden (*Analyst*, 1935, **60**, 809), that the aluminium reduction method led to results for tin that were above the truth when it was applied to solutions containing antimony, or any other metals that were precipitated by aluminium.

He did not wish to criticise or question in any way the accuracy of the figures shown by the authors, but he did wish to attract attention to the unreliability of aluminium reduction as a general method for the determination of tin in the presence of other metals.

Dr. B. S. EVANS (communicated later) wrote that he thought it only fair to point out that according to the paper published by Higgs and himself and cited here, the amount of tin carried down with the precipitated antimony and determined on the filtered out precipitate, which was carefully washed with dilute hydrochloric acid, could be as high as 8 or 9 per cent. of the amount present.

Mr. BAGSHAWE, in reply, thanked Mr. Okell for his remarks. He was aware of the unfavourable opinions of the aluminium antimony reduction method expressed by earlier workers. He confirmed the variable reducibility shown by metallic antimony and attributed this to variations of mechanical grading. This was amply demonstrated by the work of Evans and Higgs. With chemically precipitated antimony as in the present method the case was rather different as the chemical reactivity of the precipitated metal would be uniform. One would, therefore, expect that if antimony interference was a potent factor that definitely high results would be the rule. No such evidence had ever been noted either in the present investigation or by the B.I.S.R.A. Committee responsible for the initial draft relative to simple steels.

The parallel with the experience of earlier workers was hardly a good one, as the operating conditions were very different, especially the level of tin concentration under examination and the relative amounts of the reducing metal. This was also important in relation to suggestions that positive error might be partly compensated by loss of tin carried down by the precipitated antimony. Evans and Higgs had cited instances where the tin lost in this way could be as much as 8 or 9 per cent. of the amount present. In the present authors' opinion the average loss was much lower, even at relatively high tin concentrations. With lower tin concentrations the percentage loss would be lower and, in the tin ranges covered by steel, error arising from this source was insignificant.

Analysis of High-Speed Tool Steels by the Spekker Absorptiometer

Determination of the Major Alloying Constituents: Tungsten, Chromium, Vanadium, Cobalt and Molybdenum

BY GEORGE J. LENNARD

THE introduction of absorptiometric methods of analysis, using a Spekker absorptiometer, into the writer's laboratory was found to effect a great saving of time, labour and materials in the analysis of carbon and low-alloy steels. As a considerable number of the materials to be analysed are high-speed tool steels, mostly of the 18/4/1 type, it was considered desirable to adopt absorptiometric methods for these also.

It became apparent at once that the current methods for the determination of chromium based on oxidation to Cr^{VI} by ammonium persulphate in presence of silver nitrate, and for vanadium by oxidation with hydrogen peroxide, were inapplicable, owing to interference of the strongly coloured, yellow, phosphovanadotungstate complex. For the time being, therefore, the tungsten was separated by acid digestion and the filtrate was used for the determination of the above mentioned elements. It was then found that with high vanadium (4 per cent.) tool steels, chromium inhibited the formation of the pervanadic acid.

With the methods for molybdenum and cobalt no interference was detected and the procedure of Haywood and Wood[1] has been adopted. Allowance has been made, however, in the choice of sample weights to accommodate the percentage ranges commonly met with.

No satisfactory published method for tungsten was found. The hydroquinone - sulphuric acid[2] method was not considered suitable in a routine laboratory staffed with unskilled apprentices.

Finally, procedures have been worked out for the determination of chromium, tungsten and vanadium without any prior separations. The method for chromium is an adaptation of Vignal's method, whilst for tungsten and vanadium the phosphovanadotungstate complex is used.[6]

The interference of other alloying constituents with each method was determined by adding appropriate quantities of a standard solution of the element in question to a sample of a carbon steel containing negligible quantities of alloying elements and treating it according to the standard method. The results are shown in Table I.

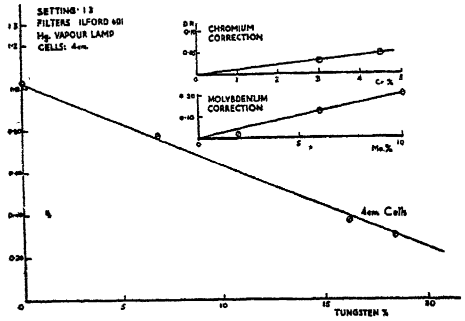

Fig. 1. Determination of Tungsten

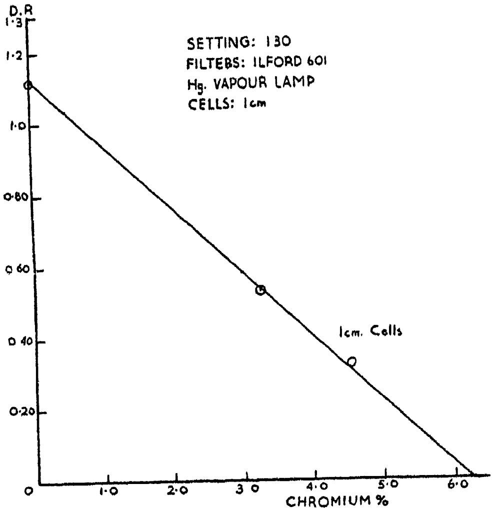

Fig. 2. Determination of Chromium

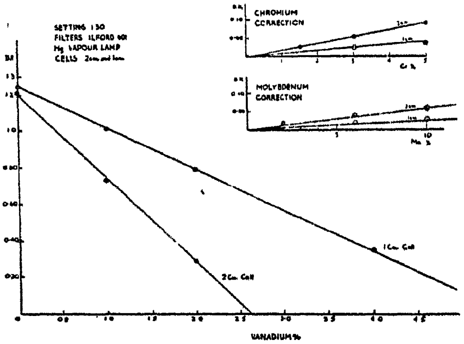

Fig. 3. Determination of Vanadium

TABLE I

INTERFERENCES OF ALLOYING ELEMENTS

Element to be determined	Other elements present Tungsten	Chromium	Vanadium	Tungsten and vanadium	Cobalt	Molybdenum
Tungsten ..	—	Construct correction graph	None	—	None	Construct correction graph
Chromium ..	None	—	None	None	None	None
Vanadium ..	None	Construct correction graph	—	—	None	Construct correction graph
Cobalt ..	None	None	None	None	—	None
Molybdenum	None	None	None	None	None	—

The absorptiometer is operated and calibrated according to the procedure of Vaughan[3,4], details of which may also be found in Haywood and Wood's monograph,[1] Chapter III. A mercury vapour lamp has been used throughout.

DETERMINATION OF TUNGSTEN (5 to 22 per cent.)

The sample is dissolved in a sulphuric - phosphoric acid mixture, oxidised with sodium nitrate and allowed to simmer to decompose carbides. The vanadium is oxidised to V^{v} by dilute potassium permanganate solution, and ammonium vanadate and dilute phosphoric acid are added to form the complex, which is stabilised by boiling. The calibration is made by using accurately standardised samples of steels of various tungsten contents. Chromium and molybdenum must be allowed for by making a correction graph.

Method—Dissolve 0·5 g. of the sample in sulphuric - phosphoric acid mixture (300 ml. of syrupy phosphoric acid and 150 ml. of sulphuric acid (1·84) diluted to 1 litre). Add one sodium nitrate Analoid (0·5 g.) and allow to simmer till carbides are decomposed. The conditions and time of simmering should be accurately controlled and just sufficient to ensure complete solution of carbides. On no account must the solution be allowed to fume. Cool and make up to 100 ml. To a 10 ml. aliquot add $N/30$ potassium permanganate until a faint pink just persists and then 20 ml. of 0·5 per cent. ammonium vanadate solution and 5 ml. of 15 per cent. phosphoric acid. Dilute to 70 to 80 ml. and boil for 10 minutes. Cool, make up to 100 ml. and determine the absorption, using Ilford Spectrum Violet filters (601) and a drum setting of 1·3. A standard should be run with every batch.

Range: With 4-cm. cells, 5 to 22 per cent.

DETERMINATION OF CHROMIUM

The sample is dissolved in dilute sulphuric acid, oxidised with nitric acid and allowed to simmer to decompose carbides. The chromium is then oxidised by boiling with permanganate. After cooling and diluting to a given volume, enough solution to fill a cell is filtered off. None of the usual alloying elements interferes.

Method—Dissolve 0·5 g. of the sample in 50 ml. of 20 per cent. sulphuric acid. Oxidise with 5 ml. of concentrated nitric acid and allow to simmer gently until all carbides are decomposed. Dilute to approximately 90 ml., heat to the boiling-point and add, dropwise, 2·5 per cent. potassium permanganate solution until chromium appears to be completely oxidised. Add a few drops of permanganate solution in excess and boil for 10 to 15 minutes. All the permanganate should now be decomposed; if not, add a few milligrams of solid manganese sulphate and boil again. Cool the solution, make up to 100 ml. and filter through a dry No. 40 paper sufficient to fill a 1-cm. cell. Determine the absorption, using Ilford Spectrum Violet filters (601) and a drum setting of 1·3.

Range: With 1-cm. cells, 0 to 6 per cent.

DETERMINATION OF VANADIUM

The sample is dissolved in sulphuric - phosphoric acid mixture and oxidised with sodium nitrate. The vanadium is oxidised to V^{v} by potassium permanganate in the cold. Sodium tungstate and phosphoric acid are added to develop the complex, which is stabilised by boiling. Chromium and molybdenum are allowed for by constructing a correction graph.

Method—Dissolve 0·5 g. of the sample in 20 ml. of "Spekker acid" (150 ml. of sulphuric acid and 150 ml. of phosphoric acid made up to 1 litre), oxidise with one sodium nitrate Analoid (0·5 g.) and evaporate to fuming. Cool and dilute to 100 ml. Take a 20-ml. aliquot and add $N/30$ potassium permanganate until a faint pink colour persists and add 5 ml. of 16·5 per cent. sodium tungstate solution and 10 ml. of 15 per cent. phosphoric acid. Dilute to 70 to 80 ml., boil gently for 10 minutes, cool and make up to 100 ml. Determine the absorption, using Ilford Spectrum Violet filters (601) and a drum setting of 1·3.

Range: With 2-cm. cells, 0 to 2·5 per cent.; with 1-cm. cells, 0 to 5·0 per cent.

DETERMINATION OF COBALT

Cobalt may be determined by the Nitroso-R-Salt method of Haywood and Wood. None of the usual alloying elements interferes.

The following conditions are recommended for the ranges usually encountered.

Range %	Sample weight g.	Aliquot ml.	Volume of Nitroso-R-Salt solution ml.	Cell size cm.
0–0·75	0·5	10	10	2
0–2·75	0·5	10	20	½
0–6·5	0·2	10	20	½
0–12·5	0·1	10	20	½
0–25	0·1	5	20	½

DETERMINATION OF MOLYBDENUM

Molybdenum may be determined by the thiocyanate method of Vaughan with small modifications in the quantities used. None of the usual alloying elements interferes.

Method—Dissolve 0·5 g. of the sample in 20 ml. of "Spekker acid," oxidise with one sodium nitrate Analoid (0·5 g.) and evaporate to fuming. Fume gently for a few minutes, making sure that all nitric acid is removed. Cool and dilute to 100 ml. Take a 20-ml. aliquot, add 20 ml. of sulphuric - perchloric acid (125 ml. of concentrated sulphuric acid, 50 ml. of 72 per cent. perchloric acid, made up to 1 litre), two sodium thiocyanate Analoids (0·5 g.) and 10 ml. of stannous chloride solution (10 g. of stannous chloride dissolved in 5 ml. of hydrochloric acid, diluted to 100 ml.). Allow to stand for 20 minutes and determine the absorption, using Ilford Spectrum Green filters (604) and a drum setting of 1·0.

Range: With 2-cm. cells, 0 to 0·6 per cent.; 1-cm. cells, 0 to 1·2 per cent.; ½-cm. cells, 0 to 2·4 per cent.

For higher molybdenum contents use proportionately smaller samples, making up the weight to 0·5 g. with pure iron.

Range %	Sample weight g.	Cell size cm.
0 to 5·0	0·25	½
0 to 12·0	0·10	½

The determinations of vanadium, cobalt and molybdenum may be done on a single sample, since the method of dissolving is the same.

When done in batches the time taken for each determination is 10 to 15 minutes. Furthermore, a complete analysis for the essential constituents, *i.e.*, carbon, manganese (by the silver nitrate - persulphate method), chromium, vanadium, tungsten, cobalt and molybdenum, may be completed in 1 to 1½ hours.

The following figures were obtained on three high-speed tool steels.

	Br. Chem. Std. No. 167		Br. Chem. Std. No. 220		Lab. Std. 18/4/1	
	Result	Standard analysis	Result	Standard analysis	Result	Standard analysis
Chromium	3·36	3·34	4·64	4·61	4·15	4·23
Vanadium ..	0·84	0·82	1·39	1·35	1·12	1·14
Tungsten ..	16·2	16·12	6·40	6·74	18·80	18·40
Cobalt ..	4·34	4·35	0·68	0·66	—	—
Molybdenum	0·53	0·55	4·22	4·17	—	—

It will be seen, therefore, that the accuracy compares favourably with that of other absorptiometric methods; at the same time there is a considerable saving in time and materials compared with the standard gravimetric and volumetric methods.

SUMMARY

Investigation into the current methods of steel analysis by means of the Spekker absorptiometer for determining chromium and vanadium in high-speed tool steels showed considerable interference due to the formation of a yellow phosphovanadotungstate complex. No satisfactory colorimetric method for the determination of tungsten could be found.

New methods for these elements have been evolved, those for tungsten and vanadium being based on the phosphovanadotungstate complex whilst for chromium the Vignal method has been adopted.

In the determination of cobalt and molybdenum no interference from the usual quantities of alloying elements was found.

With these methods a considerable saving of time and materials can be effected.

The author wishes to thank Mr. K. J. B. Wolfe, M.Sc., Chief Metallurgist, B.S.A. Tools Ltd., for his interest in this work, and Mr. J. E. MacLaren, Managing Director, B.S.A. Tools Ltd., for permission to publish this paper.

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Birmingham

June, 1948

Estimation of Grease in Sewage Sludges

By R. J. STEPHENSON

Since the digestion of sludge has been extensively practised the estimation of the grease content has become important, as the amount present is the main source of sludge gas in the earlier stages of the operation. Many methods have been proposed and two have been officially recommended in this country.[1] Much work has been devoted to the estimation of grease, particularly in the United States, where a committee was set up in 1941 to report on this subject. This Grease Referee Committee published its findings in 1945.[2] Grease was defined as "that material which is extracted from an acidified sample of sewage by petroleum ether (b.p. 40° to 60° C.) when using the standard procedure as outlined by the committee" and a large number of methods was examined, as a result of which the present standard method approved by the American Public Health Association[3] was adopted.

The methods may be divided broadly into two classes, dry extraction and wet extraction methods, both involving acidification of the sample with mineral acid. The dry extraction methods, widely used, suffer from the serious disadvantage that during the drying of the acidified sludge some loss of extractable matter inevitably occurs by steam distillation and volatisation; on the other hand, wet methods are in general slow and tedious.

The new method to be described was derived from one that has been used for some years in the Central Laboratory of the London County Council. This latter technique was introduced by Mr. J. H. Barker[4] for the purpose of estimating fat in samples of meals intended for human consumption. In this method the food sample is solidified by mixing with anhydrous sodium sulphate and allowing to stand overnight. The mixture is then ground and extracted with ethyl ether. For sewage sludges, however, Gehm and Trubnick[5] have presented several reasons for preferring light petroleum to other solvents, and light petroleum of b.p. 40° to 60° C. is the solvent used in standard American practice for the extraction of grease from acidified sewage sludges. Since the transition point of sodium sulphate decahydrate to anhydrous sodium sulphate is 32·5° C., however, the use of sodium sulphate was found to be unsatisfactory when light petroleum was used as the solvent for extraction.

Preliminary experiments showed that only part of the grease could be extracted with light petroleum of boiling range 50° to 60° C., from sludges dehydrated with sodium sulphate, as the sodium sulphate crystals liberated moisture during the extraction process. It was found, however, that if, after the first extraction, the residue was dried in an oven, ground, and again extracted, a further quantity of grease could be recovered. The total quantity of grease so obtained was greater than that which was obtained from an equal amount of the sludge that had been acidified, dried by heat, and extracted in the usual manner. It was thought, therefore, that a substance with properties similar to those of sodium sulphate, but which formed a more stable hydrate, might give very good results.

Magnesium sulphate monohydrate was chosen as a likely substance for investigation, and was prepared by heating a thin layer of heptahydrate to 97° C. overnight. The conversion was found to be almost quantitative, three 100-g. samples of magnesium sulphate heptahydrate losing, respectively, 43·0, 43·0 and 43·1 g. (Theoretical 43·8 g.) The monohydrate will readily take up three-quarters of its own weight of water. In practice, 25 g. were used to dehydrate 20 g. of sludge, as this was found to be the largest quantity of sludge that could be rapidly and satisfactorily solidified. Magnesium sulphate heptahydrate has a transition point (around 150° C.) well above the boiling-point of the light petroleum used. The solidified mixture does not sinter or liberate appreciable moisture during the extraction process. Heat is generated on mixing the monohydrate with sludge, but the temperature has been found not to rise above 67° C. during any experiment, even with laboratory temperatures as high as 30° C. The use of anhydrous magnesium sulphate, which is more difficult to prepare, was not investigated, as the considerably higher temperature rise which could be expected would not be desirable. The question of purchasing magnesium sulphate monohydrate was considered; one firm was not prepared to guarantee its product to be the monohydrate. A supply from another firm was found to contain much more water than corresponded to the monohydrate. Of two bottles from this source one lost 27·3 and the other 14·8 per cent. at 97° C., whereas there should have been no loss at this temperature. In practice, these two samples were found to be completely unsuitable.

METHOD

The method finally adopted was as follows. The sludge sample (20 to 20·5 g.) was weighed out in a 150-ml. beaker and acidified with concentrated hydrochloric acid.[*] Magnesium sulphate monohydrate (25 g.) was then added and the mixture stirred to a smooth paste. The mass began to solidify after 3 to 5 minutes and was spread on the sides of the beaker to facilitate subsequent removal. In 15 to 30 minutes it was sufficiently hard to be removed and ground up in a porcelain mortar, and the powder was packed as loosely as possible in an extraction thimble (3 to 10 cm.). The last traces of powder were transferred by means of a tuft of cotton wool, which was then used to plug the top of the thimble. Extraction was carried out in a Soxhlet apparatus at a rate of 8 to 10 cycles an hour with light petroleum of boiling range 50° to 60° C. for 5 hours. The contents of the extraction flask in solution in a small quantity of light petroleum, were then transferred, by filtration through a small plug of cotton wool inserted in the stem of a small funnel, into a weighed 50 ml. conical flask. Remaining traces were washed into the flask with a small quantity of light petroleum. The solvent was then distilled off and the vapour removed from the flask by a current of air. The flask was placed in an oven at 103° C. for 15 minutes, allowed to cool and weighed.

RESULTS—

An average of the results shows that 97 per cent. of the extractable grease was removed by the first operation, provided the thimble was loosely packed. The residue in the thimble, after crushing and repacking, yielded a further small quantity of grease on a repeated extraction. A third extraction, after repacking, yielded a negligible quantity, except in a few instances where the initial grease content was unusually high. Variations in the method of packing the extraction thimble in order to obtain more complete extraction, produced no significant differences in results. In some instances only 10 g. instead of the usual 20 g. of crude sludge were taken in order that the extraction thimble would be half filled. Typical results are given in Table I.

TABLE I

EFFICIENCY OF EXTRACTION

Sludge		Wt. of extract, mg.				Percentage of total extract in first extraction
Type	Wt. taken g.	first	second	third	total	
Digested	20·535	63	3	—	66	95·5
Digested	20·115	80	1	—	81	99
Digested	20·132	84	2	—	86	97·5
Digested	20·009	80	3	—	83	96·5
Digested	20·050	68	2	—	70	97
Digested	20·038	65	3	—	68	95·5
Crude	20·023	251	8	2	261	96
Crude	20·057	346	11	3	360	96
Crude	10·171	153	2	1	156	98
Crude	10·245	246	2	1	249	99
Crude	9·986	111	4	—	115	96·5
Crude	9·979	230	5	—	235	98
Crude	10·170	89	3	—	92	97
Crude	10·033	84	2	—	86	97·5

---

[*] For the samples used in this investigation the digested sludge required 0·4 ml. and the crude sludges 0·3 ml., these additions bringing the pH to 2·0 It will be appreciated that the presence of lime or other basic substances in the sludge would render larger additions of acid necessary.

In Table II the results obtained by the new method are compared with those given by the technique recommended by the American Public Health Association. In every case the figures given by the magnesium sulphate method are comparable with those given by the American method, although they are invariably rather higher. This may be due, in the American technique, to a slight loss of grease in the colloidal filtrate produced after the sludge has been boiled and cooled, but more so in the heat drying operation. In order to make the comparison more accurate, all the sludges used were passed through a 30-mesh sieve to break up the coarser particles.

TABLE II

COMPARISON WITH AMERICAN STANDARD METHOD

			Grease in sludge			
			American standard method		Magnesium sulphate method	
1948	Type of sludge	% solids	% on wet	% calc. dry	% on wet	% calc. dry
Jan. 22	Digested	8·5	0·424	8·5	0·427	8·5
July 26	Digested	4·55	0·388	8·5	0·413	9·1
Aug. 30	Digested	5·6	0·333	5·9	0·349	6·2
Nov. 11	Digested	4·6	0·368	8·0	0·455	9·9
Feb. 5	Crude	5·0	0·893	17·8	0·915	18·3
Feb. 9	Crude	5·6	0·806	14·4	0·856	15·3
Sept. 15	Crude	7·6	1·41	18·5	1·53	20·1
Sept. 30	Crude	5·1	1·05	20·6	1·15	22·5

The magnesium sulphate method was also checked by recovering a quantity of grease which had been added to a sludge of known grease content. Grease from digested sludges was added to a digested sludge and similarly grease from crude sludges was used with a crude sludge. There is evidence that a substance or substances insoluble in light petroleum ether are formed on standing, possibly by polymerisation or oxidation, in grease which has been extracted from sludge. Although the deposit formed is almost insoluble in light petroleum and carbon tetrachloride, it is readily soluble in chloroform. The grease which was used in these experiments was freshly prepared by re-extraction with light petroleum to remove such insoluble substances. Attempts were made to incorporate grease with sludges that had been passed through a 60-mesh sieve, by homogenising in a cream-making machine. Only part of the total grease was recovered, as some remained as a film on the homogeniser, and this method was abandoned. It was found later that if grease was stirred briskly by hand with a warm sludge, the globules quickly became smaller and soon disappeared. When this had taken place, the mixture was allowed to cool and, whilst remaining in the same vessel, was solidified with magnesium sulphate monohydrate.

The results obtained by extracting these mixtures are shown in Table III. The grease was recovered in each instance with only a very slight loss of 1 or 2 per cent. This small deficit could not be recovered by repeated extraction; it may have been due to some change having taken place during the process of mixing and extraction. The results obtained can fairly be regarded as satisfactory.

TABLE III

RECOVERY OF GREASE ADDED TO A SLUDGE OF KNOWN GREASE CONTENT

Type of sludge	Original grease content %	Grease added %	Total grease present %	Total grease recovered %
Digested	0·533	0·527	1·060	1·041
Digested	0·349	0·905	1·254	1·230
Crude	1·303	0·518	1·821	1·795
Crude	1·533	0·937	2·470	2·431
Crude	1·152	1·233	2·385	2·356

The figures obtained by the new method and the American standard method have also been compared with those by a method involving the drying of a sludge, acidified with hydrochloric acid, in an evaporating basin on a water-bath, and finally in an oven at 100° C. prior to its extraction with light petroleum. The percentage of grease extracted by this last method was invariably lower than by the others. The average figures from the results obtained in a series of experiments were as follows.

	Grease in dry sludge, per cent.		
Method	Magnesium sulphate (calculated on dry)	American standard (calculated on dry)	Sludge dried by heat
Digested sludge	9·2	8·3	5·6
Crude sludge..	18·7	17·6	15·4

CONCLUSIONS

1. The method of dehydrating sludges with magnesium sulphate monohydrate is rapid and convenient, and enables their grease content to be determined accurately by extraction with light petroleum.
2. Although the quantity of material to be extracted in this method is relatively large, 97 per cent. of the grease is taken out in one operation.
3. This method is shorter and somewhat more accurate than the method adopted by the American Public Health Association.
4. The drying of acidified sludges by heat leads to low results.
5. The results indicate that magnesium sulphate monohydrate could generally be of great use in obtaining extracts with organic solvents from mixtures containing water.

The author is indebted to colleagues at the London County Council Northern Outfall Works for useful help and criticism made during the course of this investigation, and wishes to express his thanks to Sir Allen Daley, the Medical Officer of Health of the London County Council, for the facilities afforded for the research, and to Mr. C. J. Regan, the Chemist-in-Chief, for helpful suggestions.

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Notes

THE DETERMINATION OF SMALL AMOUNTS OF ARSENIC AFTER SEPARATION AS SULPHIDE

ALTHOUGH, when practicable, distillation as trichloride provides the best separation of arsenic from other elements, it is frequently convenient, especially in metallurgical analysis, to use the sulphide separation. Even when distillation is employed, it is preferable to recover small quantities of arsenic as sulphide from the large volume of distillate prior to its determination.

As little as 1 mg. of arsenic may be determined accurately by the classical iodimetric method following separation as sulphide and dissolution of the precipitate by fuming with sulphuric acid, but for smaller quantities this method is unsatisfactory. The need for a method covering the range up to 1 mg. led to the work reported in this note.

The discovery that small quantities of arsenic sulphide could be dissolved in iodine solution resulted in the adoption of the "molybdenum blue" method. Milton and Duffield[1] have reported the conditions for the development of the colour in solutions containing iodine and sodium bicarbonate and its measurement in the "Spekker"; these were adopted without further investigation.

METHOD—Collect the arsenic sulphide precipitate produced in the usual manner, on a small pulp filter (a pad of filter paper pulp placed in the apex of the funnel, no other support being necessary) and wash as usual with diluted hydrochloric acid (1 in 50) saturated with hydrogen sulphide; finally, wash twice with cold water. Return the pulp filter and precipitate to the precipitation beaker, add 10 ml. of $0{\cdot}1\ N$ iodine solution, disintegrate the pulp by stirring and warm the solution to 50° to 60° C. Filter the iodine solution through a fresh pulp filter in the same funnel and collect the filtrate in a 50-ml. measuring flask. Add 10 ml. of $1\ N$ sodium bicarbonate solution to the pulp, pour through the filter and complete the washing with cold water. Make to volume and mix. Transfer a 5-ml. aliquot portion to a test tube and complete the determination by adding "molybdic sulphuric mixture, etc." according to the directions of Milton and Duffield.[1]

Prepare standards by mixing known amounts of sodium arsenite solution with iodine and sodium bicarbonate and developing the colour precisely as for the assays.

Although the colour was found to fade, satisfactory results were obtained by making the measurement a fixed time (10 minutes was chosen) after its development. After addition of all reagents, except the last, to a batch of assays and standards, the stannous chloride was added to each in turn at half-minute intervals.

It is advisable to include two standards with each batch of assays to check the calibration curve.

Experience with the "Spekker" for this and other colorimetric determinations suggests that rigidly standardised conditions of operation should be adopted to secure accurate results. The results of a few test analyses in which known amounts of arsenic were precipitated as sulphide and determined by the above method are given below.

Results—

Experiment	As_2O_3 taken g.	As_2O_3 found g.
1	0·00099	0·00098
2	0·00050	0·00050
3	0·00025	0·00025
4	0·00099	0·00098

REFERENCE

1. Milton, R., and Duffield, W. D., *Analyst*, 1942, **67**, 279.

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November, 1948

A SIMPLE APPARATUS FOR THE DETERMINATION OF AVAILABLE CARBON DIOXIDE IN BAKING POWDER AND THE LIKE

THE determination of available carbon dioxide in baking powder, golden raising powder and self-raising flour is indispensable in consequence of the standards that have been fixed by Food Standards Orders for the yield of available carbon dioxide in these articles.

As far as the public analyst is concerned, so long as the samples come up to the required standards the actual amount of carbon dioxide is immaterial.

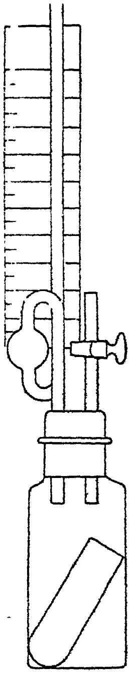
The determination of carbon dioxide and the various types of apparatus employed are too well known to need description. A simple form of apparatus for what may be called a "limit test" has obvious advantages and is here described. It is of simple construction, easily assembled and requires little attention, and the results need only an easy calculation. Its use will often avoid the more elaborate methods of determination.

It consists of a wide-mouthed stoppered bottle fitted with a tapped tube and a mercury pressure gauge, in which the weighed sample is allowed to react with water, and the amount of carbon dioxide evolved is found from the increase in pressure, the apparatus having been calibrated with sodium bicarbonate and acid.

Apparatus—

The apparatus as illustrated consists of a 4-oz. wide-mouthed bottle with a double-bored rubber cork carrying two glass tubes, one of which is a safety thistle funnel with one bulb, from which the thistle head has been removed. The inner diameter of this tube is about 5 mm., and the length of the longer arm above the cork about 10 in. Mercury is poured into this tube until the bulb is half full. The other tube, which may be straight or bent, is furnished with a glass tap. The tube inside the bottle is a cut-down test tube or sample tube, with a capacity of about 10 ml.

Attached to the mercury column by clips or wire is a paper scale marked with 100 or other divisions. Instead of the paper scale the height of the mercury column can be measured with a mm. rule.



Procedure—0·5 g. of baking powder or golden raising powder, or 5 g. of self-raising flour, accurately weighed, is transferred to the dry bottle, the inner tube, containing 7 to 8 ml. of water, carefully inserted and the cork tightly placed in position. The bottle is now stood in water at 15° C. for 5 minutes with the tap open. The level of the mercury is read and the scale adjusted if necessary. The tap is closed and the bottle removed from the water. By carefully tilting the bottle the water in the inner tube is caused to flow over the powder or flour in the bottle. The apparatus is then allowed to remain at room temperature.

With tartrated powders all the gas is generated in 1 hour, and with self-raising flours in 3 to 4 hours. With phosphated powders or flours it takes longer, sometimes 6 to 8 hours for self-raising flour, and it is better to allow it to remain overnight.

If the self-raising flour is mixed with an equal quantity of sharp sand it is wetted more readily and does not cake, and for the longer periods of standing the addition of a few drops of chloroform or thymol solution will prevent fermentation, which would give high results.

After the action has ceased the bottle is again placed in water at 15° C. for 5 minutes, the height of the mercury column is read and the amount of carbon dioxide generated calculated from the calibration curve.

Marks can be made on the paper scale to correspond with the various standards, and if satisfactory, these will avoid calculations.

According to the accuracy with which the bottle has been calibrated the process will give results varying less than 0·5 per cent. from the actual amount of available carbon dioxide in baking powder.

By using a mercury tube narrower and longer the accuracy of the determination can be improved. The solubility of the carbon dioxide in the liquid can be ignored.

The apparatus can also be used to test other articles containing aerating ingredients.

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Albert E. Parkes
April, 1948

Official Appointments

PUBLIC ANALYST APPOINTMENTS

Notification of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1949, **74**, 190).

Public Analyst	*Appointments*
Dedicoat, Herbert	County Borough of Burnley. Borough of Colne.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

Notification of the following appointment has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 191).

Official Agricultural Analyst	*Appointment*
Dedicoat, Herbert	County Borough of Burnley.

British Standards Institution

DRAFT SPECIFICATIONS

A few copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee SFE/17—Sampling and Analysis of Flue Gases.
- CK(SFE)3347—Draft Code on Indicating and Recording Instruments for Flue Gas Analysis.
- CK(SFE)3438—Draft Code on Sampling and Analysis of Flue Gases.

Draft Specification prepared by Technical Committee ELE/58—X-Ray Analysis.
- CK(ELE)3468—Draft for 9 cm. and 19 cm. X-Ray Diffraction Powder Cameras.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food & Drugs

Proximate Method for Detection and Determination of Eggs in Food Pastes. J. Kiger and R. Boivin (*Chimie Analyt.*, 1948, **30**, 172–179)—The method of Farcy (*Ann. Fals.*, 1914, 183), which depends upon the determination of nitrogen compounds soluble in hot and in cold water, is applied to the examination of pastes made with eggs, with egg-yolk powder, and with whole egg powder. The results show that the method does not lead to positive conclusions. Von Fellenberg's method (*Mitt. Lebensm. Hyg.*, 1930, **21**, 212 and 221) is even less satisfactory.

The authors' method relies on two determinations, as follows.

(*a*) *Determination of soluble nitrogen compounds precipitable by alcohol*—Powder the sample to pass a No. 50 sieve, weigh 4 g., moisten with water, dilute with water to 50 ml. in a graduated flask, and shake mechanically for 15 min. Filter, and take 7·5 ml. of the clear filtrate in a 15-ml. centrifuge tube with a tapered lower end graduated in 0·1-ml. divisions. Add 1·125 ml. of 9·3 per cent. sodium chloride solution and 6·5 ml. of 95 per cent. ethyl alcohol, shake vigorously for a few sec., and allow to stand for 48 hr. During the first 24 hr. assist the settling of the precipitate by turning and tapping the tube. The height of the precipitate after 48 hr. provides an approximate and empirical measure. To obtain an exact result, let the precipitate form over-night, and then centrifuge it in an ordinary ungraduated tube for 15 min. at 5,000 r.p.m. Decant, wash the precipitate with 15 ml. of 40 per cent. alcohol, and decant again. Transfer the precipitate, with a small amount of water, to a Kjeldahl flask and add 0·05 g. of anhydrous copper sulphate, 0·45 g. of potassium sulphate, 0·025 g. of sodium selenite, and 4 ml. of concentrated sulphuric acid. Determine the nitrogen in the usual manner, using a Parnas-Wagner apparatus. Calculate the nitrogen compounds as 5·7 times the nitrogen found.

(*b*) *Determination of ether-extractable matter*—Mix 5 g. of the sample by grinding with 5 g. of anhydrous sodium sulphate and extract the mixture for at least 5 hr. in a Soxhlet apparatus with 80 ml. of ether, to which have been added 5 ml. of liquid ammonia. Evaporate the ethereal extract in a tared Erlenmeyer flask, dry at 100° to 102° C., and weigh.

The results from these two determinations may be interpreted according to the following table.

	Soluble nitrogen compounds precipitated by alcohol		Nitrogen compounds soluble in ammoniacal ether
	Height of precipitate in ml.	Per cent.	Per cent.
Pastes without eggs	0·1–0·3	0·2–0·35	0·9–1·5
With powdered egg yolk (2 per cent.)	0·1–0·3	0·2–0·35	1·7–4·1
With 1 egg per kg. of semolina	0·4–0·5	0·5–0·6	1·7–2·1
With 2 eggs ” ” ”	0·7–0·8	0·7–0·9	2·1–2·6
With 3 eggs ” ” ”	0·9–1·0	1·0–1·2	2·8–3·2
With 4 eggs ” ” ”	1·0–1·1	1·2–1·4	3·5–3·8
With 5 eggs ” ” ”	1·2–1·3	1·4–1·7	3·9–4·3
With soya flour (20 per cent.)	less than 0·4	less than 0·45	less than 1·7
With soya albumen (10 per cent.)	0·7–1·0	1·7–1·8	less than 1·7

When the addition of soya flour or of soya albumen is suspected, it is useful to apply the Farcy (*loc. cit.*) procedure and to calculate (nitrogen compounds soluble in the cold) — (nitrogen compounds soluble hot) per cent. For ordinary pastes and for pastes made with egg-yolk powder this difference is 0·5 to 0·7 per cent.; for pastes with eggs it is 0·7 to 1·7 per cent.; and for pastes containing soya flour it can be less than 0·05 per cent. (or negative). The addition of soya albumen is shown by a high proportion of nitrogen compounds soluble in the cold. For ordinary pastes this figure is 2 per cent., and for pastes with eggs it may be up to 3·5 per cent. A paste made with 10 per cent. of soya albumen showed over 8 per cent. of nitrogen compounds soluble in the cold. W. C. JOHNSON

Observations on the Proposed Grading of Ice-Cream by a Methylene Blue Test. D. H. F. Clayson and D. G. Pirie (*J. Soc. Chem. Ind.*, 1948, **67**, 147–150)—In the tentative method proposed by the Public Health Laboratory Service of the Ministry of Health for grading ice-cream according to hygienic quality (S.R. & O., 1947, No. 612; Circular 69/47; *Monthly Bull.*, 1947, **6**, 60; *Analyst*, 1947, **72**, 153, 154), 2 ml. of ice-cream mix are added to a mixture of 7 ml. of quarter-strength Ringer solution, and 1 ml. of methylene blue solution in a test tube graduated at 10 ml., a sterile bung is inserted, the tube is inverted once, and, if necessary, the volume is adjusted to the mark with more of the sample. The mixture is incubated at 20° ± 0·5° C. for 17 hr., and finally the time of reduction of the methylene blue during incubation at 37° C. is noted.

The efficiency with which this procedure removes the air incorporated in the ice-cream depends upon whether or not the stabilising ingredient is readily dispersed on dilution. It was found that, owing to retention of liquid round the bung after inversion of the tube, the weight of ice-cream mix introduced varied from 2·45 to 2·95 g., *i.e.*, from 2·2 to 2·7 ml. of air-free mix, but that these variations do not affect appreciably the reduction times of replicates of the same sample.

When two identical series of samples were subjected simultaneously to pre-incubation at 20° ± 0·5° C. and 21° ± 0·5° C., respectively, an error of 1° C. in the pre-incubation temperature caused discrepancies of 0·5 to 1·5 hr. in the reduction times and such discrepancies may affect the grading.

The test was applied, strictly in accordance with the instructions, to 363 samples, which were also subjected to plate counts and coliform tests. Of these samples, 9 per cent. came into provisional grade 1 (reduction time 4·5 hr. or more), 68 per cent. into provisional grade 2 (2·5 to 4 hr.), and 21 per cent. into provisional grade 3 (0·5 to 2 hr.). The few remaining samples falling into provisional grade 4 (0 hr.) were from batches held up for re-pasteurisation because contamination with coliform organisms was detected by concurrent tests. The methylene blue test, however, did not prove to be a trustworthy method of detecting contamination with coliform organisms because some samples gave positive presumptive coliform tests with 0·1 ml. of the mix and occasional samples, giving positive presumptive coliform tests with 0·01 ml. of mix, came into provisional grades 2 or 3. Of the samples in provisional grade 3, 83 per cent. gave plate counts of less than 10,000 per g., over 90 per cent. showed no presumptive coliform organisms, and 60 per cent. were free from coliform organisms even after pre-incubation. This appears to be the main drawback of the test, and experience shows that such discrepancies are commoner than the report suggests.

Variations of the milk powder content of ice-cream affected the distribution of tested samples between the grades. The test was therefore applied to samples of four different batches of the milk powder. These were of low bacterial count (1400 to 8200 per g.) and contained 3 to 6 per cent. of moisture at the centre of the containers and 4 to 8·5 per cent. near the surface. Aliquots (2 ml.) of 10 per cent. solutions of the milk powders were used instead of 2-ml. aliquots of the ice-cream mix; these were each diluted with 7 ml. of quarter-strength Ringer solution and were pasteurised for 10 min. at 160° F. and cooled to 20° C. before the methylene blue solution was added. Reduction occurred in 3 to 4 hr. at 37° C. after the prescribed incubation period at 20° C., and thus milk powder alone would account for a provisional grading of 2 with only thermoduric organisms present. By emulsifying 10 per cent. of margarine in these milk powder solutions it was shown that the fat caused a further diminution in the reduction time of the order of 0·5 hr.

When 16 ice-cream samples with reduction times ranging from 0 to 3 hr. were re-pasteurised in the reductase tubes before addition of methylene blue, the reduction times ranged from 2 to 3·5 hr., *i.e.*, the samples would have been placed in provisional grades 2 or 3. Even the control tubes, which, in accordance with the P.H.S.L. instructions, were heated in boiling water for 15 min. before testing, occasionally gave reduction times as short as 3 to 4 hr. It is clear that if the P.H.L.S. methylene blue test is influenced by the presence of organisms of high heat resistance, it is not trustworthy as a criterion of compliance with the heat treatment regulations for ice-cream. Further investigation was made by inoculating sterile ice-cream mix with graded dilutions of a culture of a thermoduric organism isolated from a reduction test on ice-cream mix in which reduction occurred within 1 hr. after pre-incubation although aerobic, sporing bacilli only were present. Sterilised ice-cream mix previously diluted with quarter-strength Ringer solution as previously described was inoculated with the organism to give inocula containing from 100 to 12,000 organisms per ml. (based upon a microscopical count) in the reduction tubes. Methylene blue was then added and the P.H.L.S. procedure was followed. The results showed that small numbers of thermoduric organisms in the ice-cream mix can cause quite short reduction times in the P.H.L.S. test. It is possible that the autoclaving treatment necessary to sterilise the medium enhanced its activity as hydrogen donator. If the sterilisation treatment was not effective, reduction occurred in the uninoculated controls within 3·5 to 8 hr. after pre-incubation, consequently it appeared that the hydrogen donators present could be activated by any thermoduric organism present that was not in a dormant state. No reduction occurs if the medium is effectively sterilised, and thus the reduction is clearly catalysed by the thermoduric organism and not by any purely chemical factor.

Determinations were made of the bacterial count at the time of reduction of the methylene blue, a microscopical method being used to eliminate error due to clumping. The bacterial count at the time of reduction of the dye varied from 1 to 25 millions per ml. in the diluted mix with a mean value of 11·4 millions. Wilson (*Bacteriological Grading of Milk*, H.M.S.O., London, 1935, 229, p. 276), in a series of tests on raw and pasteurised milk with a plate count method, showed that in the presence of organisms from raw milk, reduction occurred at a mean count of about 25 millions per ml., whereas in the presence of organisms from pasteurised milk reduction occurred at mean counts of 70 to 100 millions per ml. It is therefore apparent that pasteurised ice-cream may require less catalyst in the form of bacterial cells to effect reduction of methylene blue than does pasteurised milk.

A. O. Jones

Photometric Determination of Alpha- and Beta-Amylase in Flour. E. G. Hoskam (*Biochim. et Biophys. Acta*, 1947, **1**, 419–427)—The volume of the finished rye loaf, unlike that of the wheat loaf, is not of importance, and most notice is taken of the crumb structure. Since the so-called "sticky crumb" of rye bread is attributed to the liquefying and dextrinising action of α-amylase, a method is required to determine variable amounts of α-amylase in the presence of β-amylase. The saccharogenic action of α-amylase is less pronounced than its liquefying and dextrinising power, whereas the action of β-amylase is mainly saccharogenic, although it appears to have some dextrinising properties. Only if the α-amylase content is relatively large does it serve as a criterion of the quality of the flour.

The extinction of an iodine-starch or iodine-dextrin solution is directly proportional to the starch or dextrin concentration for all wavelengths (Hanes and Cattle, *Proc. Roy. Soc.*, 1938, *B*125, 387). Erythrodextrin, the end-product of β-amylolytic breakdown of starch, can be used as a specific substrate for the determination of α-amylase (Sandstedt *et al.*, *Cereal Chem.*, 1939, **16**, 712) and the determination can be made in a simple way by measuring the extinction after addition of iodine solution.

By isolating solid erythrodextrin the separate actions of α- and β-amylase on this substrate could be studied and it was established that the subsequent attack on erythrodextrin is governed only by α-amylase, at least during the first part of the reaction. By using a buffered solubilised starch solution instead of erythrodextrin solution it was shown that the amount of starch broken down is an additive function of the amounts of α- and β-amylase present. The amount of β-amylase can thus be found by calculation.

To prepare erythrodextrin (Weber, L.E.B. Foundation, Publ. No. 25, Wageningen, 1941), stir 200 g. of flour and 500 ml. of water to an even suspension, and, after some hours, centrifuge and filter. Check the flour beforehand for absence of α-amylase (Wijsman, *De diastase beschouwd als mengsel van maltase en dextrinase*, Thesis, Amsterdam, 1889). To 1·5 litres of a 3 per cent. solubilised starch solution add 50 ml. of the flour extract, maintain the mixture at 40° C. in a thermostat, and after 24 hr. add another 50 ml. of the extract. Follow the reaction by estimating the maltose content of the mixture. If, after another 24 hr., the limit of conversion, *viz.*, 60 per cent. has been reached, boil the mixture to destroy enzymic activity, cool, add fuller's earth, and filter. Precipitate the erythrodextrin by adding 96 per cent. ethanol until the final concentration is 80 per cent. and at the same time add 15 ml. of 5 per cent. sodium chloride solution. After decantation collect the precipitate by suction, wash it successively with 80 per cent. ethanol and acetone and dry it over sulphuric acid in a vacuum desiccator. Prepare a 0·625 per cent. solution by dissolving 3·125 g. in water, adding 50 ml. of 0·2 *M* acetate buffer, diluting to 500 ml. and adding a small amount of toluene as preservative. To prepare 0·2 per cent. iodine solution add 4 ml. of *N* iodine to 5 g. of potassium iodide dissolved in water and dilute to 250 ml. To prepare the starch solution dissolve 6·25 g. of solubilised starch in boiling water, add 50 ml. of 0·2 *M* acetate buffer and dilute to 500 ml. adding a small amount of toluene. To prepare purified α-amylase extract finely-ground malted barley with water at room temperature for some hours, filter the suspension, heat the filtrate on the water-bath for 20 min. at 75° C., cool in ice-water, and remove the precipitate by suction. To the clear filtrate add ethanol to a final concentration of 80 per cent. and also some sodium chloride. After 18 hr. collect the precipitate by suction, wash first with 80 per cent. and then with 96 per cent. ethanol and dry over sulphuric acid in a vacuum desiccator. To prepare β-amylase, extract finely-ground pearl barley, free from traces of α-amylase, with water at room temperature, filter, to the clear filtrate add ethanol to a final concentration of 50 per cent., and proceed as in the preparation of α-amylase.

For determining the extinction, a Moll extinctiometer, provided on each of the sources of light with Schott glass filters OG_1 10 mm. and BG_1 10 mm. (a combination with maximum transmission at 580 mμ.), is recommended. To prepare the standard curve, for which purpose two points are sufficient, place 0·4 and 0·8 ml. of erythrodextrin solution (or 0·16 and 0·32 ml. of starch solution) in separate 100-ml. flasks containing 5 ml. of 0·2 per cent. iodine solution, and, after dilution to the mark, measure the extinctions. For the blank determination dilute 0·2 ml. of the iodine solution to 100 ml.

To prepare the enzyme, extract triturate, an accurately weighed amount (up to 6 g.) of the flour with pure sand in a mortar for 3 min. with enough water to yield a suspension that is not too thick. Transfer the suspension quantitatively to a graduated shaking cylinder and dilute to 50 ml. After mixing, place the cylinder in a thermostat at 30° C. and after 1 hr. filter through a folded filter, repeating the filtration if necessary.

Pipette 10 ml. of the substrate solution into a 100-ml. flask, place this solution and the flour extract (in another flask) in a thermostat at 30° C., and, when the flasks have attained this temperature, add 5 ml. of the extract to the substrate. Note the time by stop-watch, and after *x* min. add 1 ml. of enzyme - erythrodextrin solution (or 0·4 ml. of enzyme - starch solution) to a 100-ml. flask already containing 3 ml. of 0·2 per cent. iodine solution. Repeat this after the lapse of 2*x*, 3*x*, and 4*x* min. On the average *x* is 3 to 5 min. according to the velocity of reaction, but, with very small contents of α-amylase, it may have to be 15 to 30 min. to obtain noticeable conversion. By varying the amounts of flour in preparing the extracts it is possible to keep *x* more or less constant. After diluting to the mark measure the extinctions and, after applying the blank correction, determine the substrate concentration from the standard curve. Plot these concentrations against time and, if suitable amounts of flour have been taken, the curve will be linear.

The α-amylase activity (A^{e}_{α}) is the amount of erythrodextrin in milligrams converted per min. by the α-amylase present in 1 g. of flour. The decrease in concentration after 1 min. is directly proportional to the amount of α-amylase in the substrate. The activity has, within the limits of error of the method, a mean constant value of $19·5 \times 10^2$ mg. of erythrodextrin converted by 1 g. of sample per min.

The breakdown of starch by the combined action of α- and β-amylases was found to be a strictly additive function. For the determination of the combined activity 500 mg. of flour are sufficient. The combined activity ($A^{s}_{\alpha+\beta}$) is expressed as milligrams of starch converted per min. by 1 g. of the sample, and for about 30 rye flour samples ranged from 28 to 137, most of the values being between 50 and 100. For the α-amylase preparation the starch activity (A^{s}_{α}) was found to be $14·9 \times 10^2$, and for the β-preparation used (A^{s}_{β}) $31·3 \times 10^2$. Since the ratio of the activity of the purified α-amylase preparation towards starch to its activity towards erythrodextrin is $A^{s}_{\alpha}/A^{e}_{\alpha}$, or 14·9/19·5, or 0·76, the value of A^{s}_{α} can be calculated from that of A^{e}_{α}. A^{s}_{β} can then be found, since $A^{s}_{(\alpha+\beta)} - A^{s}_{\alpha} = A^{s}_{\beta}$. The factor (0·76 in this instance) depends upon the susceptibilities of both substrates, and has to be re-determined when new stocks of substrates are prepared.

A. O. JONES

[Semimicro-] Determination of Sulphonamides by Titration in Anhydrous Acetic Acid. O. Tomíček (*Coll. Czech. Chem. Comm.*, 1948, **13**, 116–130)—Many amino compounds, when dissolved in acetic acid, behave as strong bases and can be titrated satisfactorily with 0·1 *N* perchloric acid in acetic acid solution. Carboxyl groups introduce no difficulty, so that the amino groups of amino acids, and bases combined with carboxylic acids, can be determined by this method. The same principle is now applied to a number of sulphonamides. The titrations are followed potentiometrically, the glass, chloranil or quinhydrone electrodes being used, and the behaviour of certain visual indicators is investigated.

Apparatus—The method requires a potentiometric system of higher sensitivity than that normally used for aqueous solutions and a valve voltmeter is used throughout. The titration vessel is a 50-ml. beaker equipped with a motor-driven stirrer. For chloranil and quinhydrone procedures a platinum spiral electrode is used and the liquid is connected to a calomel half-element with a bridge tube containing a saturated solution of lithium chloride in 96 per cent. alcohol and closed with a thick, sintered-glass disc. The glass electrode is a thin glass bulb and is used with a calomel half-element with a saturated potassium chloride bridge in the form of a capillary packed with asbestos. The micro-burette is of 5-ml. capacity and is graduated in 0·02-ml. divisions. Temperature control, between 18° and 20° C., is adopted in the use of the volumetric apparatus, because of the high coefficient of expansion of acetic acid.

Perchloric acid, approximately 0·1 *N, in acetic acid solution*—Mix 5 ml. of 20 per cent. perchloric acid with 50 ml. of acetic acid and add 25 ml. of acetic anhydride *cautiously* in small portions with continual cooling and stirring. Make up to 100 ml. with acetic acid.

Sodium acetate, 0·1 *N, in acetic acid*—Dissolve 0·530 g. of anhydrous sodium carbonate in sufficient acetic acid to produce 100 ml. of solution.

The perchloric acid solution is standardised by titrating with it 5 ml. of 0·1 *N* sodium acetate in acetic acid diluted with 15 ml. of acetic acid. Sharp inflexions are obtained in curves plotted for the glass, chloranil or quinhydrone electrodes, and the end-points coincide volumetrically. At this end-point, methyl violet shows a blue-green colour and naphthol benzein a yellow-green colour, the colour change with each indicator providing a satisfactory visual end-point.

Titration curves are given for sulphanilamide, sulphamethylthiazole, sulphapyridine, 4 : 4′-diaminodiphenylsulphone and *p*-aminophenylbenzenesulphonamide. The curves refer to the glass electrode, but further data included show that the quinhydrone and chloranil electrodes are also applicable to the titration of most of the substances. Sulphapyridine and 4 : 4′-diaminodiphenylsulphone behave as di-acidic bases, the others all as mono-acidic bases, and the curves for the last three show very clear end-point inflexions. Crystal violet can be used as indicator in the titration of these sulphonamides, but α-naphthol benzein is not satisfactory.

Other substances that have been titrated by the methods described are *m*-aminobenzenesulphonamide, *p*-aminobenzene-sulphonamido-*p*′-benzenesulphon-dimethylamide, and 1-aminonaphthalene-6 and 7-sulphonamides. The last two substances and *p*-aminophenyl-benzenesulphonamide fluoresce bright blue or greenish-white in acetic acid solution, and this fluorescence becomes markedly duller at the end-point of the titration. These substances, therefore, serve as their own indicators when the titration is carried out in ultra-violet light.

W. C. JOHNSON

The Parri Reaction and the Sulphonamides. C. Lapière (*Analyt. Chim. Acta,* 1947, **1**, 390–392)—The Parri reaction (*Boll. chim. farm.,* 1924, **36**, 401; *cf.* Zwikker, *Pharm. Weekblad,* 1931, **68**, 975) is not specific for barbiturates. An intense violet colour is also obtained on adding aqueous ammonia solution to a solution of sulphathiazole and cobalt nitrate in methanol. Sulphapyridine and sulphadiazine give a much less intense violet colour, unstable in an excess of alkali. In the reaction with sulphathiazole the addition of an excess of ammonia produces a crystalline, rose coloured precipitate of the composition

$$(\text{Sulphathiazole})_2\text{Co}(\text{NH}_3).3\text{H}_2\text{O}.$$

This substance becomes blue on heating to 110° C. and then has the formula (Sulphathiazole)$_2$Co. This can be reconverted to the rose-coloured compound by dissolving in methanol or acetone and adding aqueous ammonia solution.

W. C. JOHNSON

Determination of Ephedrine in Various Medicinal Combinations. W. W. Hilty and D. T. Wilson (*J. Amer. Pharm. Assoc.,* 1948, **37**, 227–231)—Methods are described for the analysis of ephedrine salts, tablets, ampoules, and solutions, syrups and jellies of ephedrine, and ephedrine spray, and for certain medicinal preparations of which ephedrine is an ingredient.

Procedure for ephedrine salts—Dry the sample at 100° C. for 2 hr. and transfer about 0·3 g. to a 500-ml. Kjeldahl flask. Add 40 ml. of water, 25 ml. of concentrated hydrochloric acid, and boil under a reflux condenser for 1·5 hr. Cool, rinse the condenser with 50 ml. of water, and dilute the contents of the flask with water to approximately 225 ml. Connect the flask to a condenser and a receiver containing 40 ml. of 0·05 *N* sulphuric acid. Add 1 g. of zinc dust and 50 ml. of 50 per cent. w/w sodium hydroxide solution to the flask and distil until about 150 ml. of distillate have been collected. Cool the distillate and titrate the excess of acid with 0·02 *N* sodium hydroxide, using methyl red as indicator. Make a blank determination on the reagents. One ml. of 0·02 *N* sulphuric acid ≡ 0·003302 g. of anhydrous ephedrine.

Procedure for tablets of ephedrine sulphate and ephedrine hydrochloride—Reduce about 20 tablets to a fine powder, transfer an aliquot representing about 0·3 g. of ephedrine sulphate or hydrochloride to a 500-ml. Kjeldahl flask and continue as described for ephedrine salts. One ml. of 0·02 *N* sulphuric acid ≡ 0·004285 g. of ephedrine sulphate or 0·004034 g. of ephedrine hydrochloride.

Procedure for ampoules, solutions and syrup of ephedrine—Conduct the determination described for ephedrine salts on an aliquot of the preparation containing approximately 0·3 g. of ephedrine sulphate or hydrochloride.

Procedure for ephedrine jellies—Transfer an accurately weighed portion of jelly, equivalent to about 0·25 g. of ephedrine, to a 500-ml. Kjeldahl flask, and continue as described for ephedrine salts.

Procedure for ephedrine spray—Transfer a measured volume of the spray, equivalent to about 0·25 g. of ephedrine, to a 500-ml. Kjeldahl flask, and add 25 ml. of concentrated sulphuric acid. Boil under a reflux condenser for 2 hr., cool, and wash the condenser with 50 ml. of water, 50 ml. of ether, and 50 ml. of light petroleum. Mix well, transfer the mixture to a large separating funnel, and wash the flask with two 25-ml. portions of ether and two 25-ml. portions of water. Allow the mixture and washings to separate, transfer the aqueous phase to a second separator, wash it twice with 25 ml. of ether, return the aqueous solution to the Kjeldahl flask, and the washings to the original ether - light petroleum phase. Wash the combined organic solution twice with 25 ml. of water, and add the washings to the aqueous solution in the Kjeldahl flask. Warm the flask over boiling water to remove the last traces of ether, add sufficient water to dilute the contents to about 225 ml., attach a condenser and receiver containing 40 ml. of 0·05 *N* sulphuric acid, and continue the determination as previously described.

Procedure for combination of ephedrine salts and a barbiturate—Reduce 20 tablets to fine powder and transfer an aliquot equivalent to about 0·1 g. of ephedrine to a separating funnel containing 10 ml. of 2 per cent. sodium hydroxide solution, and extract with at least six successive 25-ml. quantities of ether, collecting the extracts in a second separating funnel. Wash the combined ethereal extracts three times with 15 ml. of water or until the washings are no longer alkaline to litmus. Extract the combined alkaline washings with 25 ml. of ether and add the ether to the combined ether extracts in the second separating funnel. Retain the alkaline washings. To the ethereal extracts, add 15 ml. of 0·05 *N* sulphuric acid, shake well, allow to separate, and transfer the aqueous layer to a flask. Wash the ether twice with water and add the washings to the flask. Warm the flask to remove all traces of ether and titrate the excess of acid with 0·02 *N* sodium hydroxide, using methyl red as indicator.

Combine the alkaline solution in the first separating funnel with the alkaline aqueous washings and acidify with concentrated hydrochloric acid. Extract five times with 25-ml. portions of a solvent consisting of 1 part of alcohol and 4 parts of chloroform, and collect the extracts in a second separating funnel. Wash the combined extracts with 25 ml. of 2 per cent. hydrochloric acid, filter the chloroformic solution into a tared flask, evaporate with the aid of a current of air, dry the residue at 100° C. for 15 min., cool, and weigh the barbituric acid.

Procedure for capsules containing ephedrine hydrochloride, aminophylline, and amytal—Transfer an aliquot of the mixed contents of a number of capsules, equivalent to approximately 0·25 g. of aminophylline, to a separating funnel containing 25 ml. of 2 per cent. sodium hydroxide solution, extract six times with ether, and collect the extracts in a second separator. Wash the ethereal extracts twice with 10 ml. of water, preserving the washings in a third separator. Extract the wash water with 15 ml. of ether and add the latter to the combined ether extracts and the wash water to the alkaline solution in the first separator. Extract the combined ether solutions with 10 ml. of 0·05 *N* sulphuric acid and then successively with 10 ml. and 5 ml. of water. Combine the acid and water extracts, warm gently to remove traces of ether, and titrate the excess of acid with 0·02 *N* sodium hydroxide, using methyl red as indicator.

Acidify the combined alkaline solutions in the first separator and transfer the solution, with the aid of small quantities of water. Add dilute aqueous ammonia until the solution is alkaline to litmus and then add 6 ml. in excess. Add 20 ml. of 0·1 *N* silver nitrate, allow to stand until the precipitate settles, filter and wash the filter paper with water until the washings give no reaction for silver nitrate. Transfer the filter paper and residue to the original beaker and dissolve the precipitate in 10 ml. of concentrated nitric acid. Add 50 ml. of water, cool, and titrate with 0·1 *N* ammonium thiocyanate, using ferric ammonium sulphate as indicator. One ml. of 0·1 *N* ammonium thiocyanate ≡ 0·0230 g. of aminophylline.

Transfer the filtrate just obtained to a separator and acidify it with concentrated hydrochloric acid. Extract five times with a solvent consisting of 1 part of alcohol and 4 parts of chloroform, wash the combined alcohol - chloroform extracts with 25 ml. of 2 per cent. of hydrochloric acid, discard the acid, and filter the chloroformic solution into a tared beaker. Evaporate the solvent by means of a current of air, dry the residue at 100° C. for 10 min., and weigh as amytal.

Procedure for capsules containing ephedrine hydrochloride, acetylsalicylic acid, amytal, and phenacetin—Transfer an aliquot of the mixed contents of a number of capsules equivalent to approximately 0·5 g. of phenacetin to a separating funnel containing 25 ml. of 2 per cent. of sodium hydroxide solution. Extract with six successive portions of ether and wash the combined extracts twice with 10 ml. of water. Extract the wash water with 15 ml. of ether, and add the ether to the combined ether extracts and the wash water to the alkaline solution in the first separator. Preserve this alkaline solution for the determination of acetylsalicylic acid and amytal.

Extract the combined ether solutions with 10 ml. of 0·05 *N* sulphuric acid, and then successively with 10 ml. and 5 ml. of water. Wash the combined acid and water extracts with 15 ml. of ether and wash this with 10 ml. of water. Combine the water portion with the acid extracts collected in a flask and the ether portions with the ether extracts retained in the separating funnel. Warm the acid extracts to remove traces of ether, cool, and titrate the excess acid with 0·02 *N* sodium hydroxide, using methyl red as indicator. One ml. of 0·02 *N* sodium hydroxide ≡ 0·004034 g. of ephedrine hydrochloride.

Transfer the combined ether extracts to a tared beaker, evaporate to dryness, dry in a desiccator to constant weight, and weigh as phenacetin.

Acidify the combined alkaline solutions from the first extraction with hydrochloric acid and extract five times with a solvent consisting of 1 part of alcohol and 4 parts of chloroform. Wash the alcohol - chloroform extracts with 15 ml. of 2 per cent. hydrochloric acid and discard the acid portions. Extract the chloroformic solution twice with 50 ml. of 4 per cent. sodium hydrogen carbonate solution and then with 20 ml. of water. Combine the sodium hydrogen carbonate and water extracts and extract twice with 25 ml. of chloroform, collecting these chloroformic extracts in the separating funnel containing the preserved chloroform. Filter the chloroformic extracts into a tared beaker. Evaporate the solvent by means of a current of air, dry the residue at 100° C. for 10 min., and weigh as amytal.

Transfer the sodium hydrogen carbonate solution to a flask and boil for 5 min. Add 5 ml. of 20 per cent. sodium hydroxide solution and boil for 15 min., cool, and dilute to 500 ml. with water. Transfer an aliquot equivalent to approximately 0·065 g. of acetylsalicylic acid to a glass-stoppered flask, add 25 ml. of 0·1 *N* bromine, and 6 ml. of hydrochloric acid. Set aside for 30 min. with occasional shaking, add 15 ml. of 10 per cent. potassium iodide solution, and titrate with 0·1 *N* sodium thiosulphate. One ml. of 0·01 *N* bromine ≡ 0·003 g. of acetylsalicylic acid.

A. H. A. ABBOTT

Biochemical

Chemical Assay for Crystalline Benzyl Penicillin. W. J. Mader and R. R. Buck (*Anal. Chem.*, 1948, **20**, 284–285)—The benzyl penicillin (G) content of commercial crystalline penicillin is determined by precipitation and weighing of the N-ethyl piperidine salt.

REAGENTS—*Amyl acetate solution*—Saturate pure amyl acetate with the N-ethyl piperidine salt of penicillin G by adding 200 mg. of the salt to 100 ml. of the solvent and shaking. *Phosphoric acid solution*—Dissolve 2·0 ml. of reagent-grade phosphoric acid (85 per cent.) in 8·0 ml. of water. *N-ethyl piperidine solution*—Dilute 2·0 ml. of reagent-grade N-ethyl piperidine with 8·0 ml. of pure amyl acetate; saturate the solution with the N-ethyl piperidine salt of penicillin G (about 40 mg. of salt per 10 ml. of solution). *Acetone solution*—Saturate reagent-grade acetone with the N-ethyl piperidine salt of penicillin G (about 400 mg. of salt per 100 ml. of acetone). Cool all reagents to between 0° and 8° C. for at least 30 min. before use, and filter when withdrawing by wrapping a plug of cotton wool on the tip of the pipette.

Procedure—Weigh between 50 and 75 mg. of the crystalline penicillin into a tared 10-ml. centrifuge tube. Add 2 ml. of cold distilled water and shake to dissolve. From a calibrated 2-ml. pipette add the cold amyl acetate solution, followed by 0·50 ml. of cold phosphoric acid solution. Shake well until the insoluble penicillin acid disappears. Centrifuge the tube until two layers form. Remove the amyl acetate layer by means of a hypodermic syringe with a long needle, and dry by passing over about 0·1 g. of powdered anhydrous sodium sulphate placed in a micro-filter funnel; collect the amyl acetate solution in a small test tube contained in the suction flask, and use the minimum suction to prevent evaporation. In a tared 15 × 50-mm. weighing bottle place 1 ml. of the acetone solution and 0·50 ml. of the N-ethyl piperidine reagent; add 1·0 ml. of the dried amyl acetate filtrate. Keep the weighing bottle in the refrigerator for 2 hr., and then filter the solution through a tared micro-filterstick. Wash the precipitate with a total of 1 ml. of acetone solution from a 1-ml. hypodermic syringe. Dry the weighing bottle and filterstick under vacuum at room temperature for at least 1 hr. and weigh. The percentage of sodium penicillin G in the sample is 159·3 × wt. of salt ÷ wt. of sample. For potassium penicillin G, use the factor 166·4 instead of 159·3.

For accurate analysis, keep the time between acidification and the precipitation of the salt short, preferably less than 3 min. The reagents should be tested using pure crystalline penicillin G; a recovery better than 98·5 per cent. should be obtained. Store the N-ethyl piperidine in brown bottles and protect it from carbon dioxide.

G. H. TWIGG

Spectrophotometric Method for Determination of Urinary Bilirubin. G. E. Thoma and D. M. Kitzberger (*J. Lab. Clin. Med.*, 1948, **33**, 1189)—A simple and accurate spectrophotometric method for determining urinary bilirubin depends on the production of a green colour by oxidation of bilirubin with a modified Fouchet's (acid ferric chloride) reagent. It is used at pH 4, since it was found that the original Fouchet's reagent at pH 1, and with 25 per cent. trichloroacetic acid (pH 1) in the blank, also produced a green colour developed in the blank. At pH 4 oxidation seems dependent on the ferric chloride, and does not take place in the blank.

METHOD—*Ferric chloride reagent*—6·25 g. of trichloroacetic acid and 2 ml. of 10 per cent. ferric chloride solution diluted to 200 ml. with distilled water.

Standard bilirubin solution—Dissolve 10 mg. of pure bilirubin in 100 ml. of chloroform. Add 20 ml. of this solution to 80 ml. of 95 per cent. ethanol to give a solution of bilirubin containing 2 mg. per 100 ml., which can be diluted with 95 per cent. ethanol as desired.

Preparation of standard curve—A Coleman Junior spectrophotometer is used with 15-mm. cuvettes and adapter; readings are made at a wavelength of 670 mμ. Four ml. of the chloroform - ethanol solution of bilirubin of known concentration, 4 ml. of 95 per cent. ethanol and 2 ml. of the ferric chloride reagent are placed in one cuvette. The blank consists of 4 ml. of the 20 per cent. chloroform - ethanol solution, 4 ml. of 95 per cent. ethanol and 2 ml. of 3·125 per cent. trichloroacetic acid. After 10 min. required for full development of the green colour, the spectrophotometer is set at 670 mμ., and at 100 per cent. transmission with the blank solution, and the percentage transmission of the standard solution is read. This is plotted on semi-logarithmic paper with percentage transmission as abscissa (logarithmic scale) and concentration of bilirubin in mg. per 100 ml. as ordinate.

The solution is found to obey the Beer - Lambert law; the curve is, in fact, a straight line, and can be constructed by joining the plotted point to the origin, where concentration equals 0 and percentage transmission equals 100 per cent. [The authors' graph shows 39 per cent. transmission for a solution containing 2 mg. per 100 ml.—ABSTRACTOR.]

Procedure for urine—To 4 ml. of undiluted urine add 4 ml. of 95 per cent. ethanol and 2 ml. of ferric chloride reagent. For the blank take 4 ml. of urine, 4 ml. of 95 per cent. ethanol, and 2 ml. of 3·125 per cent. trichloroacetic acid. Wait 10 min. for colour development and read as above. Bilirubin concentration is obtained from the graph. The alcohol is used to free bilirubin from protein that might be present in the urine. The only interfering substances so far encountered are salicylates, which give a pink colour with the reagent if present in sufficient concentration.

Recovery of added bilirubin in urine was from 87 to 100 per cent. Preliminary studies showed normal urine to contain less than 0·5 mg. of bilirubin per cent. A. H. BAYNES

Spectrographic Determination of Beryllium in Biological Material and in Air. J. Cholak and D. M. Hubbard (*Anal. Chem.*, 1948, **20**, 73–76)—Beryllium is isolated as the phosphate, and determined in the arc spectrograph by comparison with thallium as internal standard. Quantities down to 0·25 μg. can be detected.

Reagents—Calcium phosphate solution—Dissolve 2·5 g. of calcium carbonate in the minimum quantity of concentrated hydrochloric acid. Add 2·5 g. of diammonium hydrogen phosphate and dilute to 100 ml. with distilled water. If a precipitate forms, add hydrochloric acid to dissolve it. *Spectroscopic buffer solution*—Use any ionisable salt or salt mixture, dissolved in diluted hydrochloric acid (1 + 9) containing thallic nitrate at a concentration of 100 mg. of thallium per litre of solution.

Preparation of samples—Freshly voided urine—Place 40 ml. of urine in a 50-ml. centrifuge tube, add 0·5 ml. of the calcium phosphate solution; add concentrated aqueous ammonia dropwise until the solution is just alkaline to phenol red. Centrifuge for 3 min. at 3000 r.p.m. and discard the supernatant liquid. Dissolve the precipitate in 0·5 ml. of hydrochloric acid and transfer quantitatively to a graduated 15-ml. conical centrifuge tube. Add 1 ml. of the spectroscopic buffer solution and reduce the volume to 1 ml. by evaporation on an oil-bath at 120° to 140° C. *Animal tissues*—Place about 10 g. of tissue or blood (or 50 ml. of urine) in a 250-ml. Pyrex beaker, add 5 ml. of concentrated nitric acid; heat until charring occurs. Destroy the char by repeated addition of small amounts of nitric acid and heating (5 ml. of 70 per cent. perchloric acid may be added towards the end). When oxidation is complete, evaporate off all but a trace of the sulphuric acid. Cool, transfer the solution to a 50-ml. centrifuge tube and treat as for freshly voided urine. In digesting samples of bone, filter off the calcium sulphate before isolating the beryllium as phosphate. If iron is present in such quantity that its final concentration in the buffer would exceed 1 mg. per ml., remove it after precipitating the beryllium as phosphate. Dissolve the precipitate in hydrochloric acid, transfer the solution to a separating funnel and dilute to 20 ml. with distilled water. Add 4 ml. of a 6 per cent. cupferron solution and extract the iron with successive 10-ml. portions of chloroform until the last portion of chlorofrom added is colourless. To the aqueous phase add 1 ml. of spectroscopic buffer solution, and evaporate down to 1 ml. *Dust samples*—Dissolve in 5 per cent. nitric acid solution, evaporate the solution to a small volume and transfer it to a graduated 15-ml. conical centrifuge tube. Add a suitable quantity of spectroscopic buffer solution, and evaporate the mixture to the volume of buffer added.

Spectrographic technique—In the positive graphite electrode, make a crater 3 mm. in diameter by 10 mm. in depth, and impregnate it with 0·2 ml. of the solution; dry for 30 min. at 110° C. Turn the negative electrode to a fine point. Operate the arc at 110 v. D.C. and 10 amp. Photograph the spectrum for 2 min., using a rotating stepped sector. Measure with a non-recording densitometer the H and D curves for the beryllium line at 2348·6 A. and the thallium line at 2379·7 A. The average separation between these curves at a constant blackening value is a linear function of the logarithm of the beryllium concentration. Prepare a calibration chart by adding known amounts of beryllium to 40 ml, of distilled water and proceeding as for freshly voided urine. The most accurate analytical range lies between 0·25 and 2 μg. of beryllium per millilitre of solution.

G. H. TWIGG

Estimation of Homogentisic Acid. A. Neuberger (*Biochem. J.*, 1947, **41**, 431–437)—Three methods are described for estimating homogentisic acid, which is excreted by patients suffering from alcaptonuria. In Brigg's method (*J. Biol. Chem.*, 1922, **51**, 451) the colour that is formed in the reaction with phosphomolybdic acid takes some time to develop to its maximum and the rate of colour development depends on the concentration of homogentisic acid. The colour develops more slowly with *p*-hydroxyphenylpyruvic acid, but the difference is not sufficient for a differential estimation. Beer's law is obeyed only with lower concentrations of homogentisic acid. The reaction is not very selective and has to be used with caution. Experimental conditions that give a recovery of about 99 per cent. in water and urine are described for the iodimetric estimation. Neither amino acids such as glycine and cysteine, nor keto acids like *p*-hydroxyphenylpyruvic acid, interfere. Hydroquinone and gentisic acid behave towards iodine like homogentisic acid, whilst catechol and dopa behave irregularly and might produce misleading results if present in significant amounts.

A new method in which homogentisic acid is estimated by the reduction of silver ions at pH 4·4 is described. The method permits a very selective estimation of homogentisic acid in the presence of other reducing substances.

Silver reduction method—Gold sol reagent—Add 6 ml. of a 0·5 per cent. solution of gold chloride and 5 ml. of 0·25 *N* potassium carbonate to 250 ml. of water. Add 1 ml. of an ethereal solution of yellow phosphorus prepared by diluting a saturated solution fivefold. Allow to stand for 30 min. and boil under a reflux for 15 min. The gold sol produced is red and contains about 70 μg. of gold per ml. It is stored in the dark at 0° C. For the estimation of homogentisic acid this solution is diluted tenfold.

Procedure—To a carefully cleaned test tube or measuring cylinder add 1 ml. of 0·2 *N* acetic acid, 0·5 ml. of 0·2 *N* sodium acetate, 2 ml. of 5 per cent. gum arabic solution (filtered through Filtercel), 1 ml. of 1 per cent. silver nitrate solution, and 0·5 ml. of the diluted gold sol. Shake thoroughly, and add 1 ml. of the solution to be tested, which should not contain more than 200 μg. of homogentisic acid. Dilute to 10 ml. with water and mix. Read the gold-brown colour in a Hilger absorptiometer with a neutral filter, usually after 2 hr. A solution containing all the reagents but no homogentisic acid gives only a very slight absorption which is allowed for either by deducting the reagent control from the experimental absorption, or by placing the control solution in the compensating cup. Concentrations of between 20 and 200 μg. per 10 ml. of final solution can be estimated under these conditions. Over this range there is approximately linear correlation between concentration and absorption coefficient if the reading is made after 2 hr. A fresh set of standards of homogentisic acid should be prepared for each series of tests.

Application to urine analysis—The presence of large amounts of chlorides makes it impossible to apply the method directly to urine and the homogentisic acid must be extracted with an organic solvent as follows. Extract 2·5 ml. of acidified urine four times with 20-ml. portions of ether. The ether should be freed from peroxides, saturated with water, and kept in a dark bottle. For each extraction, shake by hand at a constant rate for exactly 2 min. Dry the combined ethereal extracts over anhydrous sodium sulphate and filter into a distilling flask. Wash the sodium sulphate, concentrate the ether solution to about 15 ml. and extract twice with 15-ml. portions and once with 10 ml. of water. Remove most of the ether by placing the combined aqueous solution in a water-bath at 60° C. for 3 to 5 min. Dilute the volume to 50 ml. and use this solution for the estimation of homogentisic acid as described above.

Results obtained with reagents prepared on different days varied within ±5 per cent. With urine, recovery of added homogentisic acid averaged 90 per cent. with a standard variation of about 3 per cent. Recovery from plasma varied between 78 and 85 per cent. The method is very selective; adrenaline, 2 : 5-dihydroxyphenylalanine, 3 : 4-dihydroxyphenylalanine, and ascorbic acid are not extracted by ether and such compounds as hydroquinone, gentisic acid, catechol, etc., react at a different rate from homogentisic acid and can be distinguished therefrom by reading the colour ratios after 1 and 2 hr. and comparing with that obtained with authentic homogentisic acid. Phenylpyruvic acid, *p*-hydroxyphenylpyruvic acid, and the lactone of 2 : 5-dihydroxyphenylpyruvic acid do not react at all with the silver reagent.

J. S. HARRISON

Estimation of Urethane (Ethyl Carbamate) in Blood. H. E. Archer, L. Chapman, E. Rhoden, and F. L. Warren (*Biochem. J.*, 1948, **42**, 58–59)—Urethane can be quantitatively hydrolysed to ethanol, ammonia, and carbonate by boiling with sodium hydroxide solution. The urethane is estimated by determining the ethanol produced.

METHOD—*Determination of volatile reducing substances in blood before alkaline hydrolysis*—Measure 10 ml. of blood filtrate into a 100-ml. flask containing a few glass beads and connected with a Jackson's condenser that has a delivery tube extended downwards for about 15 cm., dipping below the surface of a mixture of 1 ml. of 0·1 *N* potassium dichromate and 5 ml. of concentrated sulphuric acid contained in a tube of 20-ml. capacity cooled in ice. With no water cooling on the condenser, distil about 8 ml. into the oxidising mixture. Seal a glass stopper into the tube with a drop of sulphuric acid, mix the contents, and heat to 80° C. for 20 min. Cool and wash the contents of the tube into a flask with about 100 ml. of water, add 5 ml. of 5 per cent. potassium iodide solution, and titrate the liberated iodine with 0·1 *N* sodium thiosulphate. This determination usually gives a small blank value.

Determination of volatile reducing substances in blood after alkaline hydrolysis—Under a reflux condenser boil a mixture of blood filtrate and 5 ml. of 10 *N* sodium hydroxide. Stop the cooling water and distil 8 to 10 ml. into acid dichromate mixture and proceed as described above.

The difference between the dichromate reduced in the two procedures is a measure of the urethane content of the blood. One ml. of 0·1 *N* dichromate is equivalent to 2·225 mg. of urethane.

Theoretically, this method has the disadvantage that blood may contain compounds, other than urethane, that on hydrolysis with alkali may yield volatile oxidisable substances. In practice, this blank value is normally only about 3 to 5 mg. per 100 ml. of blood and does not interfere appreciably with the estimation.

J. S. HARRISON

Colorimetric Determination of Stilboestrol and Dienoestrol. F. L. Warren, F. Goulden, and A. M. Robinson (*Biochem. J.*, 1948, **42**, 151–156)—The behaviour of stilboestrol, dienoestrol, and *iso*dienoestrol in Dingemanse's antimony pentachloride reaction and Malpress's nitro reagent has been examined.

The antimony pentachloride reaction—The original description is given by Dingemanse (*Acta Brev. Neerl. Physiol.*, 1939, **9**, 118). Difficulty was experienced in obtaining reproducible results by the simple procedure of treating stilboestrol dissolved in chloroform (washed free from ethanol) with a 50 per cent. v/v solution of antimony pentachloride in the same solvent. Quantitative measurements of stilboestrol and of dienoestrol were made with a Spekker photo-electric absorptiometer, an Ilford spectrum green filter number 604 being used. Weight for weight, stilboestrol develops more colour than dienoestrol. Many stilbene derivatives develop colour with antimony pentachloride. Ethylene dichloride is a more suitable solvent than chloroform. A good commercial grade can be used without preliminary purification. Maximum colour was obtained when the final antimony pentachloride concentration lay between 1 and 4 per cent. v/v. The reagent contained 3 ml. of antimony pentachloride dissolved in 100 ml. of ethylene dichloride. For the estimation of stilboestrol or dienoestrol, add 9 ml. of freshly prepared reagent to 1 ml. of a solution of the oestrogen in ethylene dichloride. With dienoestrol, maximum colour develops immediately on mixing with the reagent. With stilboestrol, the colour increases during the first 10 min. and then reaches a maximum. Routine colour measurements are made 15 min. after mixing. Because the rate of development of maximal colour varies with temperature, the solutions are kept at 30° C. during the 15-min. period of colour development.

The Malpress nitro reaction—A detailed description of the method is given by Malpress (*Biochem. J.*, 1945, **39**, 95). The nitro reaction is comparatively non-selective both as regards substances giving the reaction and colour development. The colour with dienoestrol gives a rather general absorption at the violet end of the spectrum. An Ilford spectrum blue-green filter number 603 gave the highest values and most sensitive slope to the calibration curve. Using this filter, the sensitivity is about one-twentieth that of estimations by the antimony reagent.

Owing to atmospheric oxidation, the chromogenic power of dienoestrol and *iso*dienoestrol rapidly deteriorates when these substances are allowed to stand in solution in such solvents as chloroform and ethylene dichloride under laboratory conditions. Stilboestrol is stable.

Although added stilboestrol could be recovered from urine with approximately 100 per cent. accuracy, attempts to demonstrate the presence of significant amounts of free or conjugated stilboestrol in the urine of subjects undergoing treatment with large doses of this oestrogen were unsuccessful. J. S. Harrison

Determination of Pregnanediol in Urine for Diagnostic Purposes. D. Huber (*Biochem. J.*, **1947**, **41**, 609–611)—Although pregnanediol, which is excreted in urine as sodium pregnanediol glucuronidate, is the chief metabolite of progesterone, it is physiologically inactive and biological tests cannot be used. The present method is a modification of that of Guterman (*J. Clin. Endocrinol.*, 1944, **4**, 262) in which the pregnanediol is isolated chromatographically.

Method—*Hydrolysis and extraction*—Boil under reflux 100 ml. of urine from a 24-hr. specimen with 50 ml. of toluene and 10 ml. of concentrated hydrochloric acid for 15 min., allow to cool, and transfer to a separating funnel. Draw off the lower layer of urine and wash the upper layer of toluene and emulsion twice with 15 ml. of 0·1 *N* sodium hydroxide and twice with 15 ml. of water.

Removal of phenolic and acidic compounds—Boil the washed solution until the water is completely removed, cool to 30° to 40° C., add a solution of 10 ml. of 1·5 per cent. sodium hydroxide solution in absolute methanol, and evaporate the mixture until it is pale yellow to green-yellow in colour. Filter the hot toluene solution through a G4 Pyrex glass filter with slight suction. Wash the precipitate with 15 ml. of hot toluene and evaporate the combined filtrates to dryness on a hot-plate in a gentle stream of air. A temperature of 120° C. should not be exceeded and all traces of toluene must be removed.

Isolation of pregnanediol—Add 4 ml. of benzene to the residue and pass the solution through a 120-mm. × 10-mm. column of Merck alumina standardised according to Brockmann. Wash the column twice with 3 ml. of benzene and four times with 3 ml. of benzene containing 20 per cent. v/v of ether. Evaporate the combined four benzene - ether extracts *in vacuo*, wash the crystalline product with 1 ml. of absolute ether, and weigh. The melting-point of the product is 232° to 236° C.

Results—Recovery experiments in which known amounts of pregnanediol were added to pregnancy and menopause urine showed very nearly quantitative recovery even when the amounts present were as small as 0·6 mg. per 100 ml. of urine. A loss of about 10 per cent. is inevitable owing to the impracticability of eluting the last trace of pregnanediol from the column. The complete determination can be effected in 3·5 hr. Four to six tests can be made in one day by one person. Pregnanediol was determined in the urine of menopause patients following the injection of progesterone; the administered hormone was partially excreted as the conversion product pregnanediol, over a period of 5 to 6 days. J. S. Harrison

Bacteriology

Semi-micro Method for Testing Quaternary Ammonium Disinfectants. E. G. Klarmann and E. S. Wright (*Amer. J. Pharm.*, 1948, **120**, 146–157)—The regular F.D.A. method for determining the "phenol coefficients" of these compounds gives untrustworthy results for two reasons: (1) a representative sample of the "medication mixture" cannot be withdrawn for test owing to a condition that is set up in the mixture apparently making the bacterial suspension non-homogeneous and (2) these compounds exert a bacteriostatic effect upon the test organisms in very high dilutions even as high as 1 to 2 million, thereby conferring a false sterility upon cultures. The authors have overcome these factors by (1) using a semi-micro method in which the whole of the "medication mixture" of diluted antiseptic and bacterial suspension is taken for inoculation (the volume being only 0·5 ml.) into the culture medium, and (2) substituting for the usual F.D.A. nutrient broth one containing 1 per cent. of Bacto-Oxgall, which neutralises the bacteriostatic effect. By this technique trustworthy and consistent results, differing very considerably from those given by the F.D.A. method, are obtained. Two examples may be cited—*Quaternary Ammonium Compound B* (10 per cent.) (chemical name not given). Phenol coefficient by F.D.A. method 19 with *Eberthella typhosum* and 23 with *Staphylococcus aurens*; by the method described, not germicidal with *E. typhosum* and 1·5 with *S. aurens*. *Dodecylamine* (10 per cent.). Phenol coefficient by F.D.A. method 15 with both organisms; by the method described, 1·3 with *E. typhosum* and 0·6 with *S. aurens*.

D. R. Wood

Iodonium Compounds and their Antibacterial Activity. L. Gershenfeld and B. Witlin (*Amer. J. Pharm.*, 1948, **120**, 158–169)—In the iodonium compounds, iodine is present as part of the positive ions and in the formation of these ions iodine is multivalent. The physiological action of these compounds on frogs and dogs is to paralyse the muscles; this is of a peripheral nature, due to paralysis of the motor nerve endings (Hartman and Myer, *Ber.*, 1893, **26**, 1727). More recently, these compounds have been studied to find out whether they possess insecticidal properties. This presented the question whether when used as an aerosol spray they may combine antibacterial as well as insecticidal properties.

The following compounds were tested—diphenyliodonium chloride; bis-*p*-chlorophenyliodonium sulphate; bis-*p*-bromophenyliodonium iodide; bis-*p*-chlorophenyliodonium iodide; bis-*p*-iodophenyliodonium iodide and diphenyliodonium iodide. All showed bacteriostatic activity in powder form when tested by the F.D.A. agar plate technique (inhibition zones from 11 to 15 mm.). Only bis-*p*-chlorophenyliodonium sulphate in saturated aqueous solution (and even when diluted 1 : 4) showed bactericidal efficiency with *Staphylococcus aureus* at 37° C. within 1 min. Solutions did not reveal the presence of free iodine and addition of sodium thiosulphate was without effect on the bactericidal efficiency. Saturated solutions of all these compounds in 95 per cent. ethyl alcohol showed greater bactericidal activity than alcohol itself against *S. aureus* at 37° C. within 1 min., and the same applies to saturated solutions in alcohol containing 10 per cent. of acetone. A saturated solution of bis-*p*-chlorophenyliodonium sulphate in acetone-alcohol mixture displayed bactericidal efficiency against *Eberthella typhosum*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* within 1 min., and was capable of killing *Bacillus subtilis* and its spores in 4 hr. at 37° C., but did not kill these bacilli or spores within 240 hr. at 25° C.

D. R. Wood

Bacteriostatic Efficiency of Iodonium Compounds. Toxicity Studies of Certain Members of this Group. L. Gershenfeld and B. Witlin (*Amer. J. Pharm.*, 1948, 170–175)—The bacteriostatic efficiency of the compounds previously investigated (*cf.* preceding abstract) was tested in aqueous solutions against *Staphylococcus aureus*, *Serratia marcescens*, *Eberthella typhosum*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *B. mesentericus*, *B. megatherium* and *Streptococcus haemolyticus*.

Minimum bacteriostatic concentrations varied from 0·001 mg. per ml. for bis-*p*-chlorophenyliodonium iodide (with *S. aureus*) to 0·8 mg. per ml. for bis-*p*-chlorophenyliodonium sulphate (with *Proteus vulgaris*). Bis-*p*-chlorophenyliodonium iodide, bis-*p*-iodophenyliodonium iodide, and diphenyliodonium iodide displayed bacteriostatic properties only against the gram-positive bacteria, including spore formers. Diphenyliodonium chloride was effective against all the test organisms. Bis-*p*-chlorophenyliodonium sulphate had no effect against *E. typhosum*; and bis-*p*-bromophenyliodonium iodide none against *E. typhosum* or *E. coli*; but both compounds were effective against the other test organisms.

In the over-all picture, diphenyliodonium chloride appears as the most generally effective substance; it was bacteriostatic against *S. aureus*, *Serratia marcescens*, *Proteus vulgaris*, *B. subtilis* vegetative form and spores, *B. mesentericus* and spores, and *B. megatherium* and spores in concentration 0·007 mg. per ml., against *Str. haemolyticus* in concentration 0·009 mg. per ml., and against *E. typhosum* in concentration 0·7 mg. per ml.; *E. coli* and *P. aeruginosa* required 0·07 mg. per ml.

The solubility of diphenyliodonium chloride in water is 0·738 per cent. and that of bis-*p*-chlorophenyliodonium sulphate 0·874 per cent.; the other four compounds have solubilities ranging from only 0·0096 to 0·04 per cent.

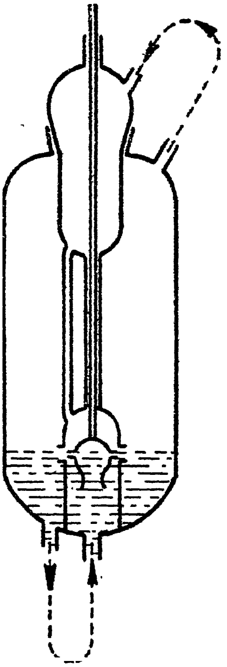
D. R. Wood

Gas Analysis

New Method for the Determination of Acetylene in Liquid Oxygen. P. A. Veldheer (*Chem. Weekblad*, 1948, **44**, 499–501)—*Procedure*—Place 500 ml. of the liquid oxygen in a conical flask, suitably lagged, and allow the gas produced by evaporation to pass through an absorption tube filled with silica gel and immersed in a liquid-air bath. When all the oxygen has evaporated, pass a current of nitrogen through the apparatus to remove the oxygen. Connect the absorption tube to a gas-washing flask with a sintered-glass diaphragm, containing 50 ml. of Ilosvay reagent. Remove the absorption tube from the liquid-air bath, and pass a current of nitrogen to transfer the adsorbed acetylene into the reagent. Filter off the precipitated copper acetylide on a filter-paper, preferably under nitrogen, and wash the precipitate with a 2·5 per cent. solution of hydroxylamine until the filtrate is free from copper. Rinse the precipitate into the point of the filter, squeeze out the moisture between the fingers, and cut off the upper part of the filter. Moisten the residue with nitric acid and ignite at 600° to 700° C. Weigh the copper oxide. It is important that all connections in the apparatus should be glass to glass, and that the nitrogen should be free from acetylene. The Ilosvay reagent is prepared as follows: Dissolve, in a 200-ml. tap funnel, 4 g. of copper nitrate in 10 ml. of water, add 16 ml. of 20 per cent. aqueous ammonia solution and 15 g. of hydroxylamine hydrochloride, and dilute to 200 ml. with water. To protect against oxidation, add a few spirals of copper wire to the solution, which can then be kept for about three days.

G. Middleton

Circulation of Gases. W. F. Engel (*Chem. Weekblad*, 1948, **44**, 511–512)—The simple apparatus described for the circulation of gases or liquids in a closed circuit is based on the centrifugal stirrer. The construction can be seen from the diagram.



With a diameter of the inner tube of 2 cm. and a speed of 1500 r.p.m., the gas flow is about 100 litres per hr. G. MIDDLETON

Water Analysis

Chlorine Dioxide in Water Treatment. A. T. Palin (*J. Inst. Water Engineers*, 1948, **2**, 61–74)—Preliminary reports suggest that treatment of water by chlorine dioxide is particularly effective for removal of taste due to phenols and algal growths. Chlorine dioxide can be produced as required by treating an aqueous solution of sodium chlorite with chlorine water, but, in order to obtain complete conversion to chlorine dioxide it is necessary to employ at least a 50 per cent. excess of chlorine. Thus chlorine dioxide, as prepared for water treatment, is always associated with free chlorine. The advantages claimed for chlorine dioxide in comparison with chlorine may be due to its greater oxidising power.

Tests for chlorine dioxide—Chlorine dioxide gives a yellow colour with acid *o*-tolidine reagent, and a solution of chlorine dioxide in distilled water behaves as does free chlorine, *i.e.*, the colour reaches a maximum in a few seconds. However, if chlorine dioxide is produced by the chlorine-sodium chlorite process, the colour develops slowly, possibly because of varying amounts of unchanged chlorite. The addition of a small amount of ammonium chloride (0·5 ml. of 0·02 per cent. ammonium chloride solution to 100 ml. of sample) does not interfere with colour development, as the free chlorine present is converted to chloramine; thus the flash reading represents only chlorine dioxide.

The above technique may be adapted to the standard *o*-tolidine-arsenite method (*Standard Methods for the Examination of Water and Sewage.* A.P.H.A., 9th Edn., 1946, p. 100) so as to determine chlorine dioxide, free chlorine, and chloramine. A brief outline of the proposed modifications is given in the table.

The readings required are obtained by taking differences, and are given in terms of available chlorine. It is essential to allow the sample to stand in contact with the arsenite for about 10 min.

Chlorine dioxide gives a colour with neutral *o*-tolidine and with *p*-aminodimethylaniline hydrochloride. Chloramine gives no colour with neutral *o*-tolidine, whilst with *p*-aminodimethylaniline colour develops very slowly. Thus, chlorine dioxide may be qualitatively distinguished from chloramine, and from free chlorine by using the ammonium chloride procedure previously described. The author's method for estimating chlorine and chloramine (*Analyst*, 1945, **70**, 203) may thus be adapted for estimating chlorine dioxide and for analysing mixtures of chlorine dioxide, chlorine, and chloramine in water. A. H. A. ABBOTT

Order in which reagents are added	Standard method readings represent	In presence of chlorine dioxide readings represent	In presence of chlorine dioxide using NH_4Cl modification, readings represent
A.OT	IS	IS	IS
OT.A	IS + Cl_2	IS + Cl_2 + ClO_2	IS + ClO_2
OT	IS + TCl_2	IS + TCl_2 + ClO_2	IS + TCl_2 + ClO_2

A—sodium arsenite.
OT—*o*-tolidine.
IS—interfering substances.

Cl_2—free chlorine.
TCl_2—total chlorine (free chlorine plus chloramine).
ClO_2—chlorine dioxide.

Organic

Estimation of Some Official Salts of Weak Organic Acids by Titration in a Non-Aqueous Medium. M. W. Green (*J. Amer. Pharm. Assoc.*, 1948, **37**, 240–242)—The potentiometric titration of a number of salts of weak organic acids, particularly sodium acetate, sodium benzoate, and sodium propionate, and of soluble barbitone, soluble phenobarbitone, sodium sulphathiazole, and sodium sulphadiazine in non-aqueous solvents has been investigated. The salts or sodium compounds were dissolved in a mixture of equal parts by volume of propylene glycol and *iso*propyl alcohol and the solutions were titrated against 0·1 *N* or

0·2 *N* perchloric acid prepared by mixing 70 per cent. perchloric acid with a sufficient volume of a mixture of equal parts of propylene glycol and *iso*propyl alcohol. The acid was standardised against 0·1 *N* alcoholic potassium hydroxide that had itself been freshly standardised with pure potassium acid phthalate; the small amount of water in the 70 per cent. perchloric acid was the only water in the system.

Dissolution of the salts was not always complete, but when the acid liberated (acetic, propionic, etc.) was sufficient to effect complete dissolution about half-way through the reaction, the titration was considered to be satisfactory. Because of the viscous nature of the solvent, the solutions were stirred mechanically.

The end-points were determined by a Beckman pH meter with calomel and glass electrodes, but later a Fisher titrimeter with the same electrodes and a cathode tube was employed. Where potentiometric results were poor, indicators, *i.e.*, methyl orange, methyl red, and thymol blue were used.

The results were compared with those obtained by the official (U.S.P.) methods: sodium acetate, sodium benzoate, sodium salicylate, soluble phenobarbitone, and sodium sulphathiazole gave favourable results. Soluble barbitone yielded poor results with the potentiometer, but good results with an indicator. Sodium sulphadiazine could not be titrated by the proposed method.

A. H. A. Abbott

Stabilised Diazonium Salts as Reagents for the Estimation of Dihydric Phenols. I. Preparation and Properties of some Diazonium Naphthalenesulphonates. II. Colorimetric Estimation of Dihydroxybenzenes, Adrenaline and 3:4-Dihydroxyphenylalanine. P. Heinrich and W. Schuler (*Helv. Chim. Acta*, 1947, **30**, 886–891; 1948, **31**, 320–330)—**I.** Because alkaline solutions of dihydric phenols are sensitive to atmospheric oxygen the investigation was directed towards diazonium compounds that give suitable colours by coupling in acid solution. To avoid difficulties that may arise through interaction of an excess of nitrous acid or of amine with other substances likely to be present, the diazonium compounds were first isolated as pure, stable α- or β-naphthalenesulphonates. Preparative methods are detailed for the following reagents and their colour reactions with certain compounds are tabulated: 4-nitro, 4-nitro-2-chloro- and 4-nitro-2-methoxy-benzenediazonium α-naphthalenesulphonates; 4-methyl-2-nitro-6-bromo-, 4-nitro-2-methoxy-5-chloro-, and 4-nitro-2-chloro-benzenediazonium β-naphthalenesulphonates. The last-named, which was used in the subsequent work, is prepared as follows.

Preparation of 4-nitro-2-chlorobenzenediazonium β-naphthalenesulphonate—Suspend 17·3 g. of 4-nitro-2-chloroaniline in 100 ml. of hydrochloric acid (d = 1·19) and 50 ml. of water, heat to boiling for a few minutes, pour the mixture on to 100 g. of crushed ice, and provide external cooling. With good stirring add a solution of 7 g. of sodium nitrite in 50 ml. of water during 0·5 hr. Filter, cool the filtrate to −5° C., and add a solution of 25 g. of β-naphthalenesulphonic acid in 100 ml. of water. Filter off the brick-red precipitate and wash it by mixing with 100 ml. of 5 per cent. hydrochloric acid and filtering. Repeat the washing, once with acid and then 5 times with 100-ml. quantities of water. Suspend the precipitate in 100 ml. of water and leave 2 hr. in the dark. Filter off and dry in a vacuum desiccator. Stir the dried and powdered product with 100 ml. of methanol, leave for 10 min., and filter. Repeat the methanol treatment and dry in a vacuum desiccator. M.p. 148° to 150° C.

The reagent gives the following colour reactions.

Substance	Colour in acid solution	Colour in alkaline solution
Phenol ..	colourless	reddish-orange
Catechol ..	yellow	violet-brown
Resorcinol ..	deep orange	violet
Quinol ..	yellowish-orange	orange
Tyrosine ..	colourless	blue-violet
Tyramine ..	colourless	pale violet
Histidine ..	colourless	orange
Histamine ..	colourless	orange
Adrenaline ..	yellow	greenish-yellow
Dihydroxy-phenylalanine	orange	brownish-red
Blank.. ..	colourless	yellow

II. 4-Nitro-2-chlorobenzenediazonium β-naphthalenesulphonate, when used according to the procedure described below, will detect 0·05 µg. of a dihydric phenol in 1 ml. No reaction is obtained with glutathione, ascorbic acid, and sympathol, which substances yield a reaction when arsenomolybdic acid is used as phenol reagent. The following give no reaction when present at less than 0·1 per cent. concentration; aliphatic and aromatic amino acids, aldehydes, ketones, cysteine, cystine, imidazole, and indole derivatives. Derivatives of pyridine and quinoline and aromatic diamines react even in low concentrations and must be removed.

When the colour intensities are measured with the Zeiss Step-photometer, using filter S43, conformity with Beer's law is shown for concentrations up to 5 µg. per ml. This is so also with the Weka photometer with Schott filter BG12, but the Lumetron photometer 400 A. with Blue 420 filter shows marked deviation from Beer's law and must be used with a calibration curve.

The colour (with adrenaline) develops more intensely as the pH increases from 1 to 3; but above pH 3 the reagent becomes sensitive to light. The most suitable temperature is 35° C. At higher temperatures the blank assumes a positive value and the reaction is less selective.

Procedure—The solution must contain not more than 5 µg. of dihydric phenol per ml. Adjust the pH to between 2 and 3 and take 10 ml. in a glass-stoppered test tube of 2·5-cm. external diameter. Add 2 ml. of a freshly prepared 0·1 per cent. solution of 4-nitro-2-chlorobenzenediazonium β-naphthalenesulphonate, previously adjusted to pH 2 to 3, and place the tube in a water-bath at 35° C. for 60 ± 3 min. As a blank use 12 ml. of the same reagent solution. Measure the colour intensity with a suitable photometer, using a blue-violet filter with a transmission range of 400 to 430 mμ. W. C. JOHNSON

Anilic Acid Number, an Analytical Index for Testing Dibasic Acid Anhydrides. C. P. A. Kappelmeier and W. R. Van Goor (*Analyt. Chim. Acta*, 1948, **2**, 146–149)—Anhydrides of dibasic acids react with aniline to form either an anil or an anilic acid. The reaction that occurs depends mainly on the solvent employed, and under standardised conditions anilic acids are quantitatively formed when aniline is added to an acetone solution of the anhydride. The anilic acid can be titrated with alcoholic potassium hydroxide solution. The number of milligrams of potassium hydroxide required to neutralise the anilic acid formed from 1 g. of dibasic acid anhydride should be known as the anilic acid number, and this index utilised as a measure of the purity of such anhydrides.

Pure dibasic acid anhydrides yield theoretical values for the anilic acid number, but higher values are obtained when a free dibasic or other carboxylic acid is present as impurity. When it is certain that only a given dibasic acid anhydride and its corresponding acid are present in a mixture the proposed determination allows the content of the two components to be easily determined from the difference between the calculated and the found anilic acid number.

The table compares the theoretical anilic numbers of typical dibasic acid anhydrides with the values obtained experimentally.

	Anilic acid number		
	Calculated	Determined	
Phthalic anhydride ..	378·4	(*a*)	378
		(*b*)	379
Tetrachloro-phthalic anhydride	195·8	(*a*)	196
		(*b*)	197
Succinic anhydride ..	560·0	(*a*)	560
		(*b*)	561
Maleic anhydride ..	571·4	(*a*)	571
		(*b*)	572
Addition product of maleic anhydride to linseed oil	121·0	(*a*)	122
		(*b*)	125
Ditto	93·0	(*a*)	92
		(*b*)	88

Procedure—Weigh accurately about 0·5 g. of the sample, dissolve it in 5 ml. of pure, neutral acetone and add 5 ml. of pure, neutral aniline. . Mix well, ignore any crystalline precipitate, and titrate immediately with 0·5 *N* alcoholic potassium hydroxide, using phenolphthalein as indicator.

With acid anhydrides of high molecular weights, use a larger sample and boil the mixture for 30 min. under a reflux condenser before titration.

Calculate the anilic acid number in the same way as a normal acid value.

Account must be taken of the acid value of oils when calculating the anilic acid number of the so-called "maleic treated oils." A. H. A. ABBOTT

Studies in Selective Extraction and Adsorption. I. Selective Solvents for Ethylene Chlorohydrin. C. Weizmann, E. Bergmann, E. F. Chandley, H. Steiner, M. Sulzbacher, and E. Zimkin. II. Isolation of Ethylene Chlorohydrin by Adsorption. M. Sulzbacher and R. Pariser (*J. Soc. Chem. Ind.*, 1948, **67**, 203–205, 205–206)—I. The isolation of pure, water-free ethylene chlorohydrin from an 8 per cent. solution in water can be achieved by addition of salt and extraction with selective solvents. The efficiency of six classes of organic solvents, *viz.*, hydrocarbons, halo- and nitro-hydrocarbons, alcohols, carbonyl compounds, esters, and ethers, has been investigated. Of these, aldehydes, ketones, and esters are the most suitable, although the efficiency varies considerably within each class. The distribution coefficient is defined as (parts of chlorohydrin in the organic layer) ÷ (parts of chlorohydrin in the aqueous layer) for equal volumes of the solvent and a saturated salt solution at 20 ± 2° C. This figure reaches a maximum of 6·5 for *cyclo*hexanone and for 3 : 5-dimethyl*cyclo*hexanone. Of the esters, ethyl acetate and *cyclo*hexyl acetate are the most effective and have each a coefficient of 4·0. The probable reasons for the variations in the behaviour of the many solvents are discussed.

II. Ethylene chlorohydrin can be isolated from its dilute aqueous solution by adsorption on active carbon and treatment of the absorbate with *cyclo*hexanone. The total amount of chlorohydrin that can be adsorbed by a given quantity of carbon is about the same for 12·0 per cent. and 7·4 per cent. w/v aqueous solutions, respectively, although the amount adsorbed varied from 35 g. per 100 g. of carbon of one brand to 57·7 g. per 100 g. of another brand. A certain amount of water is also adsorbed. The extraction was effected as follows. A current of hot air was applied to an adsorbent bed that had been treated with 400 ml. of a 12 per cent. chlorohydrin solution and which contained 37·2 g. of ethylene chlorohydrin and 80 ml. of water. Thirty grams of the chlorohydrin and the total amount of the water were collected, *i.e.*, a 27·3 per cent. w/w solution of the chlorohydrin. Twenty-five ml. of *cyclo*hexanone were then fed into the column, which adsorbed it. Renewed application of hot air released the remaining 7·2 g. of the chlorohydrin together with the *cyclo*hexanone. W. C. JOHNSON

Argentometric Micro-determination of Organic Chlorine. K. Sisido and H. Yagi (*Anal. Chem.*, 1948, **20**, 677–678)—Conversion of organically-bound chlorine to the ionic form by the Stepanow method (*Ber.*, 1906, **39**, 4056) in which metallic sodium is used requires no special apparatus, is free from explosion, and is rapid and accurate. The Volhard back-titration, which is unsuitable in this determination, is avoided by using bromophenol blue as adsorption indicator in titrating with silver nitrate solution, the colour change being sharp even in presence of alcohol and of decomposition products.

Procedure—Weigh a sample containing about 2 mg. of chlorine as in the Dumas micro-method and transfer it to a 50 to 100-ml. flask fitted with a ground-in reflux condenser, and dissolve the sample in not more than 3 ml. of *n*-butanol, *iso*-butanol, or amyl alcohol previously digested with sodium and distilled to remove aldehydes. If the sample is not easily soluble in the alcohol, use a suitable halogen-free solvent and then add the alcohol. Add between 0·03 and 0·05 g. of sodium, to maintain a fairly constant blank correction, and attach the condenser. Shake the flask until reaction ceases, then heat gently for 10 min., and finally heat vigorously under refluxing conditions for 10 to 15 min. Add 10 ml. of water to the cooled solution to dissolve sodium chloride and neutralise to bromophenol blue with 10 per cent. acetic acid (pH of the solution 3·0). Titrate with 0·01 *N* silver nitrate from a micro-burette until 1 drop changes the colour from blue-green to violet. Determine the factor of the silver nitrate by repeating the procedure without the sample and adding 5·00 ml. of 0·01 *N* sodium chloride after the digestion.

Results—On duplicate values for six samples ranging from 17 to 90 per cent. of chlorine the accuracy is within ±0·4 per cent. M. E. DALZIEL

Estimation of Thiophen in Gasoline. H. C. McKee, L. K. Herndon, and J. R. Withrow (*Anal. Chem.*, 1948, **20**, 301–303)—The blue indophenine colour produced by reaction between thiophen and isatin, in the presence of sulphuric acid, is used for the direct estimation of thiophen in gasoline without interference from other sulphur compounds.

Procedure—Add 1 drop of concentrated nitric acid to 50 ml. of a 0·04 per cent. solution of isatin in concentrated sulphuric acid, mix, add 1 ml. of the gasoline sample, and mix thoroughly. A blue colour appears almost immediately and reaches a maximum in 20 to 30 sec. Determine the transmittance of the solution in a spectrophotometer at 540, 550, and 560 mμ., and average the results obtained. Deduce the concentration of thiophen in the sample by reference to curves prepared by plotting the percentage concentration of thiophen-sulphur against the corresponding percentage transmittance for 1-ml. aliquots of standard solutions of thiophen in gasoline, containing from 0·02 to 0·5 per cent. of thiophen-sulphur. If the concentration of thiophen-sulphur in the sample is less than 0·025 per cent., use 2 ml. for the determination and divide the result read from the calibration curve by 2. In the same manner determine concentrations of thiophen-sulphur greater than 0·5 per cent. by using smaller aliquots of sample.

For each determination, measure the transmittance of a blank solution containing an equal amount of thiophen-free gasoline as the test solution, both test and blank being made up and measured at the same time.

Visual observation method—To two solutions of 50 ml. of 0·04 per cent. isatin in concentrated sulphuric acid containing 1 drop of concentrated nitric acid add sufficient of the unknown gasoline sample to produce the indophenine colour in one, and 1 ml. of a standard thiophen-gasoline solution containing approximately the amount of thiophen expected in the sample in the other. Mix each solution and compare their colour intensities under a strong light against a white background. Continue to add small measured increments of the sample, with thorough mixing after each addition, until the colour of both solutions is the same after each solution has been set aside for 5 min. Determine the amount of thiophen-sulphur present in the unknown from the formula: $C_1M_1 = C_2M_2$, where C_1 = concentration of thiophen-sulphur in the sample, M_1 = ml. of sample used, C_2 = concentration of thiophen-sulphur in the standard solution, and M_2 = ml. of standard solution used.

Because many factors affect the intensity of colour produced, results by photo-electric measurement are not always accurate to within less than 15 to 20 per cent. of the thiophen-sulphur present, whilst for visual measurement results are usually accurate to within 25 or 30 per cent. of the thiophen-sulphur present. Experience of the method greatly increases accuracy. A. H. A. ABBOTT

[Polarographic] Analysis of 1:2:3:4:5:6-Hexachloro*cyclo*hexane for the Gamma Isomer. G. Dragt (*Anal. Chem.*, 1948, **20**, 737–740)—The polarographic method described for determining the gamma isomer of 1 : 2 : 3 : 4 : 5 : 6-hexachloro*cyclo*hexane depends on the fact that of the five isomers examined, only the gamma isomer is reduced at the dropping mercury electrode. These five isomers are known to be present in technical material. Interference from heptachloro*cyclo*hexane is avoided by measuring the polarographic diffusion current by a special technique.

METHOD—*Reagents*—*Stock solution A*—Dissolve 2 g. of potassium chloride and 20 g. of sodium acetate trihydrate in 50 ml. of water, add a solution of 0·1 g. of carpenter's glue in 20 ml. of water, and dilute to 100 ml. with water. *Stock solution B*—Dissolve 0·01 g. of carpenter's glue in 20 ml. of hot water, dilute to 110 ml., add 2 g. of potassium chloride and, when dissolved, dilute to 250 ml. with 95 per cent. ethyl alcohol. *Stock solution C*—Mix 60 ml. of acetone with 40 ml. of water.

Procedure—Dissolve 0·4 g. of the sample in 80 ml. of acetone and dilute to 100 ml. with water. Transfer 10 ml. of this solution to a 50-ml. volumetric flask, add 20 ml. of solution C, and 5 ml. of solution A, and dilute to 50 ml. with solution B. Transfer a portion of this solution to a polarographic cell and pass nitrogen through the cell for 15 min. The nitrogen should be bubbled first through a sample of acetone, then through a solution of approximately the same composition as that under examination and finally through the cell, to avoid losses due to evaporation. After complete removal of dissolved oxygen, record a polarogram between −0·5 and −2·0 v. *versus* the saturated calomel electrode.

To measure the step-height, extend the straight portion of the curve between −0·6 and −1·0 v. to meet the −1·3-v. perpendicular, and from this intersection draw a line parallel to the zero current line. The perpendicular distance from this line to the curve at −1·7 v. gives the height of the step. The heptachloro*cyclo*heptane reduction step does not interfere if this procedure for measurement is followed. The concentration of the gamma isomer is read off from a calibration curve with a precision of ±0·5 per cent. J. G. Waller

Determination of Gluconuric Acid in Glycoproteins. S. I. Osaki (*Tohoku J. Exp. Med.*, 1947, **49**, 15–17)—The modified Tillmans and Philippi method (Osaki, *J. Biochem. Japan*, 1936, **24**, 73) and the Dische (*Mikrochem.*, 1929, **7**, 33) method for gluconuric acid cannot be used for glycoproteins that yield hexoses on hydrolysis, but the dyes produced by hexoses in the above reactions can be absorbed by phosphotungstic acid, whilst those produced by the gluconuric acid are not absorbed.

Procedure—Shake the well-ground sample, containing 40 to 60 mg. of gluconuric acid, with 5 ml. of 1·0 *N* sulphuric acid and heat in a sealed tube in boiling water until the substance is dissolved (1 to 2 hr.), and then for 1 hr. more. Cool, and filter. Mix 4 ml. of the filtrate with 1 ml. of a 20 per cent. w/v solution of phosphotungstic acid ($H_7[P(W_2O_7)_6].22H_2O$), set the mixture aside for 1 hr., and filter. To 1 ml. of the filtrate apply the Dische (*loc. cit.*) method. Dilute the remainder of the filtrate twice with water and use 1 ml. of the diluted fluid for the modified Tillmans and Philippi method (Osaki, *loc. cit.*). Agitate the cold reaction mixtures each with 0·5 g. of finely powdered phosphotungstic acid, transfer each, with the acid, to a Gwiggner funnel (*vide infra*) plugged with glass-wool, and filter with suction. Determine the depths of colour in the clear filtrates against standards prepared by subjecting 0·010 per cent. glucose solution to the colour reactions. Prepare calibration curves using pure gluconuric acid. If the proportion of hexose present in the determinations is excessive, the two figures obtained for gluconuric acid will not coincide. The Gwiggner funnel is a conical funnel having a 3-mm. (internal) stem widened to 4 mm. (internal) through 8 mm. of its length at the upper end. The widened part of the stem accommodates the glass-wool plug.

W. C. Johnson

New Method for the Estimation of 1 : 2-Dithiols. W. N. Aldridge (*Biochem. J.*, 1948, **42**, 52–58)—The method described is based upon the reaction between cyanogen chloride and 1 : 2-dithiols to give a compound that liberates thiocyanate in alkaline solution.

Reagents—*Cyanogen chloride solution*—(*a*) For the estimation of BAL (1 : 2-dimercaptopropanol) and other 1 : 2-dithiols in aqueous solution, pipette approximately 0·1 ml. of cyanogen chloride, using an ice-cold pipette, into 200 ml. of *N* sodium acetate. (*b*) For estimations in blood and urine, take 0·1 ml. of cyanogen chloride in 100 ml. of 3·8 per cent. sodium sulphate ($Na_2SO_4.10H_2O$) solution. Cyanogen chloride (b.p. 14° C.) is highly toxic and should be stored in a refrigerator in a bottle with a well-greased stopper having an external joint. *Bromine water*—A half-saturated solution is used for the estimation in aqueous solution and a saturated solution for blood and urine.

Procedure—To 2 ml. of a solution containing not more than 10 µg. of a 1 : 2-dithiol, add 0·4 ml. of cyanogen chloride reagent, mix, and allow to stand for 5 min. Add 0·4 ml. of *N* sodium hydroxide and allow to stand for 10 min. Make acid by adding 0·2 ml. of 3 *N* hydrochloric acid and bubble air saturated with water vapour through the solution for 30 min. Add 0·2 ml. of bromine water, mix, and remove the excess of bromine with 0·2 ml. of a 2 per cent. solution of arsenious acid (boiling is necessary to dissolve the arsenious acid completely). Mix well, blow out any bromine vapour left in the tube, and pour into a previously prepared mixture of 4 ml. of pyridine reagent (made by adding 100 ml. of concentrated hydrochloric acid to 1 litre of a constant boiling mixture of pyridine and water containing 59 per cent. of pyridine) and 0·8 ml. of benzidine reagent (5 per cent. of re-crystallised benzidine hydrochloride in 2 per cent. v/v hydrochloric acid). The total volume of solution is 8·2 ml. Mix well by pouring between the tubes. Allow the colour to develop for 20 min. and then read on a photo-electric absorptiometer with 1-cm. cells and Ilford spectrum green 604 filters. Syringe pipettes (maximum error 0·2 per cent.) are preferable, with the numerous additions of small quantities of reagents, and accuracy in measurements is essential. To prevent catalytic oxidation of the dithiols due to the presence of traces of metals, the distilled water is saturated with carbon dioxide and 10 µg. of sodium cyanide per ml. are added to poison the metallic catalysts. Calibration curves are given for 1 : 2-dimercaptopropionic acid, 1 : 2-dimercaptopropanol (BAL), and 1 : 2-propane dithiol.

Application to biological fluids—Pipette as soon as possible 1 ml. of whole blood into 1·5 ml. of cyanogen chloride reagent in isotonic sodium sulphate solution contained in a centrifuge tube. Allow to stand for 10 min., add 1 ml. more of cyanogen chloride reagent and allow to stand for 5 min. Add 0·5 ml. of 10 per cent. sodium tungstate solution, mix, and add 0·5 ml. of 0·66 *N* sulphuric acid, mix, and centrifuge for 10 min. The same procedure is satisfactory for BAL in plasma. It is essential to have the blood in contact with the cyanogen chloride as soon as possible in order to minimise losses of BAL due to decomposition. The procedure for urine is as given above for blood except that 1 ml. of *N* sodium acetate is added to the cyanogen chloride reagent before addition of the urine, and no tungstate or sulphuric acid is required. The procedure thenceforward is identical for blood and urine. To not more than 2 ml. of filtrate from blood protein precipitation or urine solution, add 0·4 ml. of *N* sodium hydroxide and allow to stand for 10 min. Acidify with 0·2 ml. of 3 *N* hydrochloric acid, make up to 2·6 ml. with water, and bubble air saturated with water vapour through the solution for 30 min. to remove cyanide. Brominate with 0·4 ml. of bromine water, removing excess with 0·4 ml. of arsenious acid solution. Mix, blow out any bromine vapour, and continue as described in the method above. For the thiocyanate blank determination the above procedure is followed, except that the cyanogen chloride reagent is replaced by the same volume of isotonic sodium sulphate solution, *i.e.*, 2·5 ml. The difference between the two determinations gives the 1 : 2-dithiol concentration (plus any interfering substances) and should be referred to the calibration curve as determined before.

In view of the instability of BAL in blood, indirect methods were used for estimations of its recovery. Degradation curves, which were extrapolated to zero time, were prepared. Recoveries of 90 to 95 per cent. can be expected from whole blood and plasma. The degradation rate in plasma and serum is higher than in whole blood. Much difficulty was encountered in estimating BAL in urine. Low recoveries were often obtained and the method as applied to urine is not satisfactory.

Of many thiol compounds tested to determine the selectivity of the method, only glutathione, ergothioneine, and thiolacetic acid interfere.

J. S. HARRISON

Quantitative Determination of Hemicellulose Constituents by Fermentation. A. H. Auernheimer, L. J. Wickerham, and L. E. Schniepp (*Anal. Chem.*, 1948, **20**, 876–877)—The method depends on the determination of the reducing sugars of hydrolysates of hemicelluloses from agricultural residues before and after selective fermentation of the D-xylose and of the L-arabinose present.

Procedure—To isolate the hemicelluloses from corn cobs, for example, extract the ground (4-mesh) sample at room temperature with 8 per cent. sodium hydroxide solution, precipitate the "xylan" by adding an equal volume of 95 per cent. alcohol to the extract, and separate it by decantation and filtration. Purify it by successive extractions with 50 per cent. alcohol, a 2 per cent. solution of sodium hydroxide in 50 per cent. alcohol, 50 to 66 per cent. alcohol (three times), 95 per cent. alcohol, absolute alcohol, and dry ether. With wheat straw, obtain an extract by circulating 5 per cent. sodium hydroxide solution through the straw for 25 hr., and after the final washing, acidify with acetic acid, and dehydrate as described above. The colour is less easily removed than from the corn cobs. To hydrolyse the hemicelluloses, heat under reflux a solution of 1 g. of the xylan in 3 per cent. sulphuric acid for 2 hr., cool, filter, neutralise with barium hydroxide solution to approximately pH 7·0, and filter off the barium sulphate. Evaporate the filtrates to dryness under reduced pressure, dissolve out the sugars from the residue by repeated extractions with hot absolute alcohol, evaporate the extracts to dryness *in vacuo*, and dissolve the residue in water. To aliquot portions (*e.g.*, 25 ml., equivalent to 50 to 100 mg. of total reducing sugars) of the resulting solution (which is now free from uronic acids), in 300-ml. conical flasks, add 15 ml. of an extract obtained by autoclaving 150 g. of starch-free baker's yeast with 1 litre of water at 15 lb. per sq. in. for 30 min., cooling, and filtering. Sterilise the mixture at 15 lb. per sq. in. for 15 min. Cool, and inoculate separate samples with 10 ml. of a standard suspension of *Hansenula suaveolens* (N.R.R.L., No. 838), or of *Candida guilliermondii* (N.R.R.L., No. 488) prepared by the method of Wise and Appling (*Ind. Eng. Chem.*, *Anal. Ed.*, 1944, **16**, 28), so as to give approximately 35 × 10^6 cells per ml. (as estimated photometrically). The former organism ferments selectively the D-xylose, whilst the latter ferments both this and the L-arabinose present. Ferment for 24 hr. at 30° C., with shaking, dilute to 100 ml., and centrifuge. Determine the reducing sugars (by Somogyi's method) in the decanted liquid, heating the samples for 15 min. to ensure complete reaction of the arabinose. Reducing values found are: D-xylose, 0·129; D-glucose, 0·130; L-arabinose, 0·146; and D-arabinose, 0·178 mg. of sugar per ml. of 0·005 *N* sodium thiosulphate solution. Make all fermentations in duplicate, and run uninoculated controls.

J. GRANT

Determination of Free Carbon in Compounded Rubber and Synthetic Elastomers. G. D. Louth (*Anal. Chem.*, 1948, **20**, 717–719)—A procedure for estimating the free carbon (carbon black and/or graphite) in rubber is described. Unlike previous methods it is applicable to compounded and vulcanised synthetic as well as natural rubbers. The rubber is softened in boiling tetrachloroethane and then decomposed by boiling nitric acid. The mixture is extracted with ether, the carbon remaining in the raffinate, which is then dispersed with acetone and filtered, washed, dried, and weighed. The free carbon is given by the loss in weight on ignition.

Procedure—Prepare the sample for analysis by one of the standard methods (*e.g.*, *Amer. Soc. Testing Mat.*, *Standards on Rubber Products*). Set an electric hot-plate so that the surface temperature remains between 170° and 180° C. Weigh 0·1 to 0·15 g. of the prepared sample into a 150-ml. beaker. Add 10 ml. of tetrachloroethane, insert a glass stirring rod and cover the beaker with an Erlenmeyer flask filled with cold water to prevent excessive loss of the solvent. Set the beaker on the hot-plate and heat until the sample is well softened. Add to the contents of the beaker 15 ml. of boiling nitric acid (sp. gr. 1·42) and replace the covering flask. Keep the mixture simmering until the decomposition of the sample and dispersion of the black are complete. At the end of this digestion period, cool the beaker to below 20° C. with an ice-bath, and then add approximately 25 ml. of diethyl ether with quick stirring. Allow the beaker to stand only until two layers form and then decant the upper layer through a prepared Gooch crucible under suction. Retain the lower layer containing the carbon black in the beaker. Repeat the stirring and decanting with 25-ml. portions of ether until the upper layer is colourless. Immediately following the final decantation of ether, wash down the sides of the beaker with 5 ml. of acetone delivered from a wash bottle, and stir thoroughly. Wash the Gooch crucible with ether and then reduce suction until the ether just drains through. Fill the crucible about three-quarters full with ether and then slowly pour the contents of the beaker into the crucible. To prevent re-dispersion of carbon black at least 2 parts of ether to each part of acetone should be maintained within the crucible. This can be assisted, if necessary, by a reservoir of ether connected to a capillary tube dipping into the Gooch crucible at the required level. Wash the beaker with 5 ml. more of acetone, add the washings to the crucible, which is then sucked dry. Dry the crucible at 250° to 300° C. for 30 min., cool, and weigh. Burn off the carbon black in a muffle furnace at 900° C., cool, and weigh. Calculate the percentage of free carbon from the loss in weight on ignition.

Results obtained—The reagents used are claimed not to affect the recovery of free carbon when treated in the presence of vulcanised rubber, and hence a correction factor is unnecessary. A selection from the results given for analyses of vulcanised compounded rubbers of known composition is given in the table.

DETERMINATION OF FREE CARBON IN VULCANISED RUBBERS

Polymer	Number of determinations	Free carbon present %	Free carbon found %	Mean deviation of results
Butyl	12	30·6	30·95	±0·20
Natural rubber	2	28·0	27·90	0·10
	2	32·0	31·80	0·20
GR–S (butadiene - styrene co-polymer)	2	31·0	30·65	0·25
	6	28·25	27·90	0·30
Neoprene GN (polychloroprene)	6	23·55	23·70	0·15
	6	23·55	23·70	0·25

W. C. WAKE

Inorganic

Determination of Free Silica (Quartz) in Silicates. D. Florentin and M. Heros (*Bull. Soc. Chim.*, 1947, 213–215 M)—The determination of free quartz in silicates is important as quartz is the primary constituent causing silicosis. Of the many procedures investigated, the following are recommended.

Enumeration of quartz particles in silicate dusts—Using a microscope, make a count of the number of particles in a sample of the dust. Cover the sample with tetrahydronaphthalene, which, having the same refractive index as quartz, renders the quartz particles invisible, and make a count of the remaining visible particles. Calculate the percentage of quartz particles in the sample.

Determination of free quartz in silicates (*chemical method*)—The procedure is a modification of the method of Trostel and Wille (*J. Amer. Ceramic Soc.*, 1940, **23**, 18). Fuse 1 to 2 g. of sample, ground to pass a 150-mesh sieve, with 10 to 15 g. of potassium hydrogen sulphate, using a temperature low enough to prevent appreciable loss of sulphur trioxide. Finally, heat to 1000° C., cool, and extract the melt with 150 to 200 ml. of hot water. Add in small quantities 12 g. of sodium hydroxide to dissolve the silica liberated from decomposed silicates, and keep the solution at about 90° C. for 30 min. Filter off the quartz residue, washing with hot water, ignite, and repeat the fusion with potassium bisulphate to ensure complete decomposition of silicates. Extract with water, add sodium hydroxide, and keep at 90° C. as before. Filter, washing with hot water, then with diluted hydrochloric acid (1 + 3), and finally with hot water, ignite, and weigh. If the sample is rich in iron, treat the ignited quartz residue with concentrated hydrochloric acid on the water-bath, evaporate to dryness, take up in dilute hydrochloric acid and filter, wash, ignite, and weigh. Check the purity of the quartz residue by treatment with hydrofluoric acid in the usual manner.

Determination of free quartz in silicates (*physical method*)—The method is essentially that of Trombe (*Compt. rend.*, 1938, **207**, 1110) and depends on the liberation of heat due to the transition of α-quartz to β-quartz at 573° C. The apparatus consists of a cylindrical copper heating block containing three symmetrically placed cylindrical holes. One hole contains an inert powder (*i.e.*, containing no quartz) in which is inserted a platinum - platinum - iridium thermo-couple for observing the temperature of the block. The other two holes are filled with inert material and the crushed sample, respectively, and contain identical chromel - alumel thermo-couples, which are connected in opposition through a sensitive galvanometer. The galvanometer reading is then a measure of the temperature difference between the inert material and the sample. An external heating coil is used for heating the copper block.

To carry out the determination, heat the copper block at a rate of 5° to 8° C. per min. and plot the galvanometer reading against the temperature as indicated by the platinum - platinum - iridium thermo-couple for the range 500° to 600° C. The galvanometer reading changes sign at the transition temperature, producing a curve with a minimum and a maximum, and the difference between the points of maximum and minimum in terms of galvanometer reading is a measure of the percentage of quartz in the sample. Curves using pure quartz and known dilutions of quartz must be prepared by an identical procedure for standardisation purposes.

This method gives results in close agreement with those obtained by the chemical method.

H. J. CLULEY

Semi-micro Gravimetric and Volumetric Determination of Silica. C. Duval (*Analyt. Chim. Acta*, 1947, **1**, 33–37)—Silica in colloidal form or as SiO_3'' ion easily forms, in acid medium, silicomolybdic acid, and this reacts with a number of organic and inorganic bases to give a yellow precipitate, more or less insoluble, having the general formula $Si(Mo_2O_7)_6.H_8(B)_4.4H_2O$. The base, *B*, may be hexamethylene tetramine ("hexamine"), used in the method to be described, pyridine, 8-hydroxyquinoline, etc.

The sample should contain not more than 10 mg. of silica.

METHOD—Place 5 ml. of the solution in a 100-ml. Pyrex flask with 1 ml. of freshly prepared 10 per cent. ammonium molybdate solution, add glacial acetic acid to pH 3 (bromothymol blue paper), stopper the flask, and heat for 10 min. in a water-bath so that the temperature of the liquid does not exceed 65° C. Cool, add 2 ml. of 10 per cent. hexamine solution, and set aside in an ice-box for 15 min. The yellow precipitate,

$$Si(Mo_2O_7)_6.H_8(C_6H_{12}N_4)_4.4H_2O$$

has a formula weight of 2492.

Gravimetric procedure—Filter the precipitate on an ashless paper, wash three times with 10 ml. of 1 per cent. hexamine solution, then with 5 ml. of absolute alcohol, dry at 100° C., and, to avoid loss of MoO_3, ignite at a temperature not exceeding 600° C. At this temperature the organic matter is destroyed, leaving a white residue of $SiO_2 + 12MoO_3$, formula weight = 1788. The error is about 1 in 200 when the quantity of silica exceeds 2 mg. For 0·1 to 2 mg. of silica, the technique described in *Anorganische Mikrogewichts-Analyse*, Hecht and Donau (Vienna, 1940), is suitable, the process being carried out in a porcelain crucible with a porcelain filter-stick. This procedure is applicable to ternary glasses (Si, Ca, Na), mineral waters, and vegetable ash. Phosphate, arsenate, and titanium dioxide interfere.

Volumetric procedure—The hexamine can be completely hydrolysed in acid medium to formaldehyde and ammonia, the former reacting with sodium bisulphite, the excess of which can be oxidised with iodine. The formaldehyde - bisulphite compound can be decomposed with a base and titrated with iodine. As a result, 1 mol. of silica is equivalent to 4 mol. of hexamine or 1 mg. of silica corresponds to 8 ml. of 0·1 *N* iodine.

Filter the precipitate on a small, No. 3, sintered-glass crucible, or Gooch with a disc of ashless filter paper, or by means of a filter-stick and micro-beaker. Place the crucible in a 300-ml. distillation flask together with 50 ml. of water and 1·5 to 2·0 g. of tartaric acid, pass 500 ml. of steam and collect the distillate in a conical flask containing 25 ml. of reagent sodium bisulphite (5 ml. of the commercial solution in 200 ml. of distilled water). Add starch paste and run in iodine solution to a blue colour; ignore the volume. Add 2 to 3 g. of powdered sodium bicarbonate, shake vigorously, and titrate with iodine solution very slowly until the blue colour persists. The determination takes about 2 hr., and the error is about 1 in 100. Alternatively, a known excess of hexamine can be added and, ignoring the volume of the precipitate, the excess determined in the filtrate. H. K. DEAN

Separation of Vanadium and Boron. G. Weiss and P. Blum (*Bull. Soc. Chim.*, 1947, 1077–1079 M)—The classical method for separating boron from the other elements by means of a methyl alcohol distillation is inapplicable to the separation of boron and vanadium. Brophy (*J. Amer. Chem. Soc.*, 1925, **47**, 1856) described a method for separating boron from tungsten based on the differences in solubilities of barium tungstate (insoluble) and barium borate (soluble) in presence of an excess of baryta. The authors have adapted this process to the separation of vanadium and boron.

Procedure—Weigh 100 to 200 mg. of sample into a small nickel crucible and mix with about 2 g. of sodium peroxide. Heat the mixture over a very small white flame for 20 min. and then raise the temperature somewhat for 2 min. more to frit the mass.

Extract the cooled melt with water and boil for a few min. to decompose the excess of peroxide. Precipitate the vanadium as barium vanadate by adding about 20 ml. of concentrated barium hydroxide solution and boiling for 1 min. Allow the solution to cool and settle, filter, and wash the precipitate with warm water. Reserve the filtrate for the determination of the boron.

Re-dissolve the precipitate through the filter paper with a small amount of warm, diluted nitric acid and oxidise the solution thus obtained with perchloric acid. (Alternatively, use boiling diluted sulphuric acid as solvent, but avoid attack on the filter paper by dissolution of the precipitate in a beaker.) Add an excess of standard ferrous ammonium sulphate and titrate back with 0·1 *N* potassium dichromate, using diphenylamine as internal indicator. The barium sulphate precipitate that forms in the solution does not appreciably mask the end-point of the titration.

Determination of boron in the filtrate from the precipitation of the barium pyrovanadate—Neutralise the cold solution with hydrochloric acid, using, methyl orange as indicator, and then make it slightly alkaline with barium hydroxide solution. Concentrate the liquid to about 50 ml. on a sand-bath, re-acidify with hydrochloric acid, transfer to an Erlenmeyer flask connected to a reflux condenser and boil under reduced pressure for about 20 min. to remove dissolved carbon dioxide completely.

Rinse the condenser with a small amount of water and carefully neutralise the solution and rinsings with 0·1 *N* baryta. Add 7 to 10 g. of mannitol and titrate the boric acid with the same solution.

Standardise the baryta against a standard boric acid solution, carrying out the titration as is described for the test. P. M. C. PROFFITT

Titration of Cerous Ions with Permanganate. G. Goffart (*Analyt. Chim. Acta*, 1948, **2**, 140–145)—The titration of cerous ions with potassium permanganate has been investigated by potentiometric and amperometric methods, of which the latter gave the better results. A platinum wire indicator electrode and a silver - silver chloride reference electrode were connected through a 1000-ohm resistance, and the current flowing through the circuit was observed during the titration.

Procedure—Adjust approximately 200 ml. of a saturated solution of sodium pyrophosphate to pH 7·0 to 8·0 with hydrochloric acid, and then add 25 ml. of cerous nitrate solution containing approximately 150 mg. of cerium. Lower the electrodes into the solution and titrate with a 0·02 *M* solution of potassium permanganate. At the end-point there is a sharp increase in the current flowing.

The only element that interferes is manganese.

J. G. Waller

Potentiometric Investigation of Tripyrophosphatomanganic Acid. J. I. Watters and I. M. Kolthoff (*J. Amer. Chem. Soc.*, 1948, **70**, 2455–2460)—In the presence of pyrophosphoric acid, bivalent and tervalent manganese ions are converted to stable complex ions that form a reversible oxidation-reduction system. A method is described for the determination of manganous ions, involving their conversion to the tervalent complex, which is titrated with ferrous sulphate. The titration is followed electrometrically.

Procedure for solutions containing bivalent manganese ions—Make the solution approximately 0·4 *M* with respect to pyrophosphate by adding a solution of sodium or potassium pyrophosphate adjusted to pH 2·0 with sulphuric or nitric acid. Shake the solution with lead dioxide to oxidise the bivalent complex to the tervalent state and, after filtering off the lead dioxide, titrate the solution with a standard solution of ferrous sulphate. Measure the potential difference between a bright platinum electrode and a saturated calomel reference electrode, and plot these values against the volume of ferrous sulphate solution added. At the end-point there is a sharp change in potential.

J. G. Waller

Determination of Alumina in Iron Ore. G. F. Smith and F. W. Cagle, jun. (*Anal. Chem.*, 1948, **20**, 574–576)—The method depends on the formation of a ferrous-dipyridine complex, which is not precipitated by aqueous ammonia or by ammonium carbonate. Ten milligrams of alumina can be separated from 90 mg. of ferric oxide in one precipitation.

Procedure—Take 1 g. of ore and dehydrate the silica twice with 15 ml. of concentrated hydrochloric acid. Remove the silica by hydrofluoric acid, fuse the residue with sodium hydrogen sulphate, dissolve the fused mass in water, and add the solution to the filtrate from the silica. Dilute to 300 ml., add a 25 per cent. excess of sodium bisulphite, and warm. When reduction is complete, cool, and stir in a 10 per cent. excess of 2 : 2′-dipyridine in 15 to 20 ml. of ethanol. If thiocyanate has been used to test the reduction a precipitate is now formed and must be filtered off. Add a slight excess of a saturated solution of ammonium carbonate and filter with slight suction and the addition of filter-paper pulp. Wash with 2 per cent. ammonium nitrate solution, neutralised to methyl red with ammonium carbonate, until the red colour of the ferrous-dipyridine complex ion is removed. Ignite in platinum. If calcium is present, add 5 g. of ammonium chloride before the ammonium carbonate, or dissolve the alumina precipitate in dilute hydrochloric acid and re-precipitate it by aqueous ammonia.

The 2 : 2′-dipyridine can be recovered by treating the filtrate from the alumina with sodium hydroxide and steam-distilling.

C. F. Herbert

New Modification of the Thiocyanate Method of Determining Molybdenum. L. B. Ginzburg and Yu. Yu. Lurye (*Zavod. Lab.*, 1948, 14, 538–545)—The use of stannous chloride in the absorptiometric thiocyanate method for molybdenum has the disadvantage that reduction may go beyond the Mo^{V} stage, causing the colour intensity to decrease rapidly. Potassium iodide is preferable as reducing agent since, in the presence of sodium sulphite to react with the iodine formed, the reduction goes completely and solely to the quinquevalent stage.

With 0·10 mg. of molybdenum in 50 ml. of a solution containing 4 per cent. of potassium iodide and 0·12 *N* with respect to potassium thiocyanate the optimum hydrochloric acid concentration is 3 to 4 *N*, the colour attains its maximum intensity in 2 to 3 min., and it is then stable for 1 hr. With 2 *N* acid the maximum is attained only after 45 min., and with 5 *N* and 6 *N* acid the maximum intensity is not reached at all and the colour rapidly becomes pale. The presence of sulphate equivalent to 0·3 to 0·9 *N* sulphuric acid reduces the reaction rate and it is necessary to wait for 15 to 30 min. before measuring the absorption.

Large amounts of iron have no effect on the colour intensity, but to avoid the need for excessively large amounts of iodide not more than 10 mg. of iron should be present in 50 ml. of solution.

Variations in excess of 1 per cent. of the concentration of potassium iodide have no effect in absence of iron, but even with 2 mg. of iron per 50 ml. the iodide concentration must be at least 2 per cent. to avoid delay in reaching maximum colour intensity. The use of a green filter prevents the interference due to bismuth (yellow colour) and vanadium (yellow-green colour). Tungsten and antimony solutions remain colourless on addition of the reagents. Copper is removed as cuprous iodide precipitated by potassium iodide.

Under the conditions used the sensitivity with a Klett - Summerson photocolorimeter was 0·04 mg. of molybdenum per litre. The error did not exceed 1 part in 200 in the concentration range of 2 to 12 mg. per litre.

Procedure—Dissolve 0·1 to 0·5 g. of sample, *e.g.*, molybdenum-containing ore, in 10 to 15 ml. of *aqua regia*, then add 10 ml. of diluted sulphuric acid (1 + 1) and evaporate to fuming. Cool, dissolve the salts in 30 to 40 ml. of water, transfer the solution to a 100-ml. graduated flask, dilute to the mark, and mix. Place a suitable aliquot portion containing between 0·06 and 0·6 mg. of molybdenum in a 50-ml. graduated flask or cylinder, and add to it 35 ml. of diluted hydrochloric acid (1 + 3), 3 ml. of 20 per cent. potassium thiocyanate solution, 1 g. of potassium iodide, and 1 ml. of freshly-prepared 1 per cent. sodium sulphite solution, stirring the solution after each addition. Dilute to the mark with diluted hydrochloric acid (1 + 3), and obtain the colour intensity after 10 min., using a green filter. Carry out a blank and determine the molybdenum content from a calibration curve. G. S. Smith

Thermal Behaviour of Analytical Precipitates. T. Duval and C. Duval (*Analyt. Chim. Acta*, 1948, **2**, 205–209)—*Caesium*—The anhydrous chloride is stable between 110° and 877° C. The perchlorate can be dried below 611° C., decomposition to the chloride occurring between 611° and 677° C. The sulphate is dehydrated by heating between 105° and 876° C. The monohydrated caesium cobaltinitrite decomposes on heating, but between 219° and 494° C., a mixture of [CoO + $3CsNO_3$], formula weight 659·7, which can be used as a weighing form, is produced; further decomposition occurs above 494° C. The stannichloride, Cs_2SnCl_6, is dehydrated between 130° and 344° C., but at 879° C. a further range of constant weight is reached. The platinichloride is dehydrated between 200° and 409° C.; decomposition begins above 490° C., but is not complete even at 1060° C. M. E. Dalziel

Thermal Behaviour of Analytical Precipitates. T. Duval and C. Duval (*Analyt. Chim. Acta*, 1948, **2**, 218–221, 222–225, 226–229)—*Lanthanum*—The hydroxide is completely converted into La_2O_3 above 944° C., a basic carbonate, $La_2O_3.CO_2$, being formed between 370° and 467° C. The oxalate does not reach constant weight below 876° C., above which temperature the oxide is formed. The oxinate should be dried at 130° C.; charring occurs at higher temperatures.

Cerium—The dioxide is stable between 450° and 814° C., and the anhydrous sulphate between 277° and 845° C. The double iodate, $2Ce(IO_3)_4.KIO_3.8H_2O$, is not a useful form for precipitation, nor is the double ferrocyanide $KCe[Fe(CN)_6].4H_2O$. The oxalate is precipitated as a mixture of hydrated forms, but CeO_2 is obtained on heating it between 450° and 881° C. The oxinate is precipitated as the dihydrate, and is dehydrated between 128° and 233° C.

Neodymium—The gelatinous hydroxide dehydrates gradually, the oxide Nd_2O_3 not being obtained below 608° C. The oxalate does not give a stable hydrate, but the anhydrous salt is obtained between 290° and 350° C., and conversion to oxide, Nd_2O_3, is complete above 813° C.

Samarium—Ignition of the hydroxide gives the oxide Sm_2O_3 above 813° C. The oxalate gives Sm_2O_3 above 800° C. M. E. Dalziel

Physical Methods, Apparatus, etc.

Simplified Instrument for Wide-Range Dielectric Constant Measurement. R. B. Fischer (*Anal. Chem.*, 1947, **19**, 835–837)—With the instrument described, dielectric constants from 1 to 7 are measured by a conventional capacity method, and dielectric constants from 1 to 80 by an inductance method. The instrument makes possible an extended use of dielectric constant measurements in the analysis of liquids.

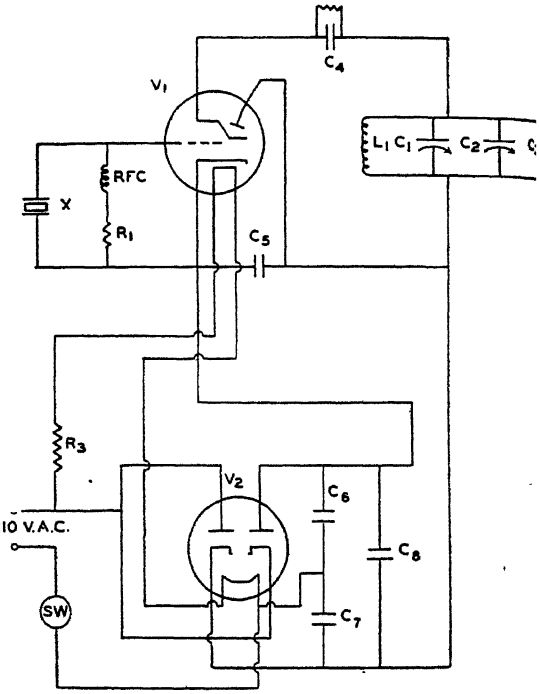


Fig. 1. Circuit Diagram of Instrument

R_1. 40,000-ohm resistor.
R_2. 150,000-ohm resistor.
R_3. 290-ohm line cord resistor.
SW. Single-pole, single-throw toggle switch.
X. Quartz oscillating crystal, about 3,500 kilocycles.
RFC. Small radio frequency choke.
V_1. Type 6E5 electron-ray tube.
V_2. Type 25Z6GT rectifier tube.
C_1. 150-mmfd. straight-line capacity variable condenser.
C_2. 10-mmfd. straight-line capacity variable condenser.
C_3. 15-mmfd. variable condenser mounted flexibly for use in small beaker as dielectric cell.
C_4. 0·001-mfd. fixed condenser.
C_5. 0·01-mfd. fixed condenser.
C_6, C_7, C_8. 16-mfd. electrolytic condensers, 450-volt.
L_1. 38 turns of cotton-covered wire wound on lower portion of $\frac{3}{4}$ inch diameter test tube.

Apparatus—The circuit diagram is given in Fig. 1. A voltage doubler rectifier system provides a plate and target voltage of about 250 v. for the 6E5 electron-ray tube. The triode oscillator circuit of the electron tube is of the tuned-grid, tuned-plate type with a piezo-active quartz crystal as the frequency controlling device. The circuit oscillates whenever the resonant circuit, L_1, C_1, C_2, C_3, is tuned to the same frequency as the quartz crystal. The condition for this is $f = 1/2\pi\sqrt{LC}$, where f is the crystal frequency, L is the inductance, and C is the capacity of the resonant circuit. The manner in which the circuit goes in and out of oscillation as the capacity of the resonant circuit is changed is shown by the curve in Fig. 2. The state of oscillation is marked by a decrease in the plate current and in the shadow angle of the "magic eye." The point at which the "eye" suddenly opens as the condenser capacity is increased may conveniently be used as a reference point in all measurements.

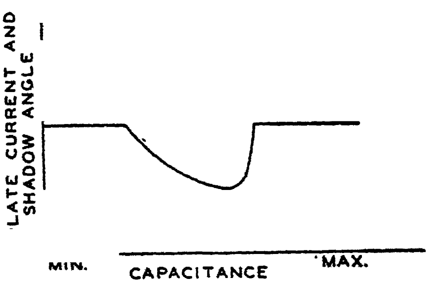


Fig. 2. Oscillator Curve.

Capacity method—Measure the capacity of C_3 in terms of C_1 and C_2, with air as dielectric and with the liquid as dielectric. With liquids of dielectric constant over 7 the total capacity of the cell is too large for oscillator characteristics to be sharp.

Inductance method—The inductance of the coil L is altered by placing the liquid in the test tube on which the coil is wound. Under the conditions used the value of this change in inductance depends solely on the dielectric constant of the liquid. The circuit is returned to oscillation by altering the capacity of the condensers C_1 and C_2. The liquid should extend over the length of the coil winding and it is advisable to use the same quantity of liquid in any series of measurements. Calibrate the instrument with a series of liquids having suitable dielectric constants and plot a graph of dielectric constant against the change in capacity of the condensers needed to bring the oscillator back into resonance. If a calibration curve over the range of dielectric constants 0 to 80 is required, distilled water, 50 per cent. ethanol in water, ethanol, amyl alcohol, and benzene are suitable standards. Over any range of 10 units the calibration curve is practically linear. [ABSTRACTOR'S NOTE—Only non-conducting liquids have been used in the apparatus.] B. ATKINSON

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal Chem.*, 1948, **20**, 879–880)—Data for β-pyridine-sulphonic acid are given (*cf. Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

Reviews

FOOD-PLANT SANITATION. By MILTON E. PARKER. Pp. ix + 447. New York and London: McGraw-Hill Book Company Inc. 1948. Price $6.00; 36s.

The feature of this stimulating book that should commend itself to most readers is that, whereas there is already an abundance of literature on the subject scattered about under individual chapter headings in books on food technology or as articles in bulletins, pamphlets and scientific journals, this book is one of the few publications with all the information under one cover. The author states that the book is intended for "food manufacturers, warehouse operators, industrial sanitarians, food technicians and those concerned with the official supervision and inspection of food industries, as well as teachers, students, research workers and to some extent the general public."

The author may be forgiven, therefore, for not going deeply into some subjects, *e.g.*, the chemical and bacteriological tests required to control and to assist sanitation, and the chemistry of water softening or of detergency although, if it were his intention only to touch the fringe of such subjects, he has not been entirely consistent, because, for example, the bacteriology of packaging materials, the toxicity of pigments for wrapping materials and the chemistry of germicides and fungicides have been discussed in more detail than his intention warrants. On the whole, however, he has covered a wide range of subjects without dwelling too long on any one.

It may surprise many people when they read this book to learn, not only the importance attached by the American public to hygiene and sanitation in the production and service of foodstuffs, but also the support to this attitude by American food manufacturers and caterers, originally resulting from the enactment of the Federal Food, Drug and Cosmetic Act of 1938, but now freely given because of the appreciation of the value of good sanitation to sales, publicity and consumer choice. As it appears inevitable that both the law and public opinion in Britain will advance in the same direction, it is increasingly important that employees at all levels in food factories should become "sanitation conscious" and for this reason this book should be of very general interest.

This theme is discussed (from the American angle) in Chapter 1 (Role of Sanitary Practice); construction experts are catered for in Chapter 2 (Sanitary Aspects of Buildings and Equipment); engineers should derive interest from this chapter and from Chapters 3, 4, 12 and 13 (Water Supplies, Waste Disposal

Detergency and Cleaning Operations); pest controllers should study Chapters 9 and 10; management, Chapters 5, 6 and 7 (Establishment of, Training in and Maintenance of Sanitary Practices); and so on. Chapters 5 to 7 are of particular interest, wherein methods are discussed for obtaining the co-operation and enthusiasm of employees, for educating them as to the reasons for trying to attain a high standard of hygiene, both personal and general, and the methods by which such standards may be maintained. Basic principles of sanitation to be laid down by the management; detailed sanitary practices to be adopted by workers; co-operation and assistance to be given by engineers, chemists and "sanitarians"; all are discussed in a stimulating manner.

Particularly useful, if only as a guide or as a basis for discussion, are the various schedules and specifications (official or otherwise), such as "Standards for Sanitary Facilities for Staff" (p. 107), "Inspection Form for Eating and Drinking Establishments—U.S. Public Health Service" (p. 154), "Inspection Technique" (p. 120) and "Laboratory Aids and Sampling Techniques" (p. 124).

This book has a wealth of detail but few references, and is quite readable except for American spelling and expressions, in places, that jar on English readers, and for a number of printer's errors that one would not expect in what is otherwise a good quality production.

P. J. NORMAN

METHODS OF QUANTITATIVE MICRO-ANALYSIS. Collected and edited by R. F. MILTON and W. A. WATERS. Pp. viii + 599. London: Edward Arnold & Co. Price 60s.

The modern method of compiling an authoritative work by obtaining contributions from a number of authors has now been applied to micro-analysis and the result is a more coherent whole than is often obtained in this way. Milton and Waters deserve praise, not only for their own considerable writing, but also for their achievement in making the best use of the other contributors, G. Ingram, J. T. Stock and K. M. Wilson. The authorship of each part of the text is indicated in the six lists of section headings which are printed at the beginning of each of the main parts into which the book is divided. The inconvenience of this scattering of the key headings is much reduced by their repetition in condensed form in the Contents on pages vii and viii.

The six parts of the book are as follows—I. Gravimetric Apparatus and General Micro-Chemical Techniques, 44 pp. II. Micro-Analysis of Organic Compounds, 70 pp. III. Volumetric Analysis, 89 pp. IV. Colorimetric Analysis, 180 pp. V. Electro-Chemical Methods of Micro-Analysis, 97 pp. VI. Gasometric Methods of Micro-Analysis, 79 pp. The editors claim that, with few exceptions, the book covers all branches of analytical chemistry, the most noteworthy omissions being spectrographic analysis and the comparatively recent microbiological assays. The former requires costly apparatus and both techniques differ from customary macro-and micro-analyses; their omission may, therefore, be excused but some of the other missing subjects, noted below, might well have been included. Only micro-methods are described; semi-micro-analysis is justifiably ignored (there are many books on this technique), but it would have been at least topical to include the ultra-micro procedures of Benedetti - Pichler which have been applied by Seaborg to the atomic energy problem.

The editors' 7-page Introduction includes a useful summary of the errors that may arise in micro-analysis, but the importance of obtaining a "fair sample" is not mentioned: without truly representative material any analysis must be worthless, and efficient sampling of industrial products is generally much more difficult than the preparation of a pure chemical compound. It is disappointing that no aspect of sampling is discussed.

Part I gives a good account of general micro-technique and, like the whole of the book, is primarily concerned with practical manipulations, attention being drawn to pitfalls and to the accuracy of the methods described. Physical determinations are not included, nor are extraction, distillation and fractionation. Nor are these subjects to be found in Part II, in which molecular weight determinations are dismissed with descriptions of Rast's and Barger's methods. Again, the valuable melting-point and mixed melting-point determinations (*e.g.*, on the microscope hot-stage) are omitted. The reference to troublesome co-precipitation and the useful "collectors" might also have shown the value of the latter in connection with recent applications of radio-active tracers.

Part III is a useful account of the technique and applications of micro-volumetric analysis. Here it would have been helpful to draw attention to Conway's excellent treatment of volumetric errors (*Micro-Diffusion Methods and Volumetric Error*), although this receives passing mention in the Introduction.

The longest section (IV) of the book is devoted to colorimetric methods. After an account of the principles involved and the apparatus available, the author gives a valuable selection of colorimetric, nephelometric and fluorimetric methods drawn from a very wide field. This part contains a 1-page description of chromatography that can serve little purpose other than to draw attention to some of the other publications on this important subject. The remaining two parts appear to the reviewer to be the most valuable, because micro-electrical and micro-gasometric methods are not described as fully or as well in any other book, although the former have been reviewed in *The Analyst* and the latter at one of the Microchemistry Group's meetings.

In pointing out the above shortcomings the reviewer has considered the book as presenting the whole of quantitative micro-analysis in a single volume, the editors' declared intention. The result has been to obscure the very real value of the material which is here collected in this form for the first time—no other work known to the writer aspires to what this work does in fact achieve. Chemists who find it difficult to produce the necessary three pounds should reflect upon the cost of the alternative of purchasing separate books covering each section of the subject.

G. H. WYATT

MAY, 1949 Vol. 74, No. 878

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, May 4th, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1, with the President, Mr. George Taylor, in the chair. The following papers were presented and discussed: "The Colorimetric Determination of Streptomycin B (Mannosido-streptomycin)," by W. B. Emery, B.Sc., F.R.I.C., and A. D. Walker, B.Sc., A.R.I.C.; "The Chemical Determination of Nicotinic Acid in Food Products," by P. O. Dennis, B.Sc., A.R.I.C., and H. G. Rees, B.Sc., Ph.D., A.R.C.S., F.R.I.C.; "The Reduction of Antimonial Tin Solutions with Metallic Nickel and Cobalt," by H. Holness, M.Sc., F.R.I.C.

NEW MEMBERS

James David Becket; Peter Rowson Booth, B.Sc. (Lond.); John Leonard Bunce; Alfred Henry Coombes, B.Sc. (Lond.), F.R.I.C.; Hubert Ian Henstock, A.R.I.C.; Robert Oliver Villiers Lloyd, B.A., M.Sc. (Dublin); Stanley Harold VanSickle.

The Polarographic Analysis of Light Metals and Alloys: A Survey

By W. STROSS

(Lecture delivered at a meeting of the Physical Methods Group organised by the Polarographic Discussion Panel on April 9th, 1948, at Leeds)

No clear definition seems to exist as to where lightness of metals ends and where heaviness begins. There is hardly any doubt that aluminium is a light metal and zinc is not, but no rule or even usage seems to exist about metals with specific gravities between these two. In the following, the limit will be drawn at the specific gravity of barium, 3·5, but a few remarks about the rare earth metals will be included.

It is intended to concentrate upon less known and less accessible publications; a number of well known monographs[7,29,40] and papers,[18,27,30] and the writer's own papers[31,32,33,34] on the determination of zinc and lead in aluminium alloys will be referred to only in the bibliography.

In this survey of methods for the analysis of light metals and alloys the determination of alloying constituents and impurities alone is being considered, not the determination of the base metal. Thus the section "Magnesium" deals with the determination of impurities or alloying constituents, which themselves may be light or heavy metals, in magnesium metal or magnesium-based alloys.

The estimation of the base metal is rarely carried out in metallurgical practice, even by gravimetric or volumetric methods; polarographic methods are, generally speaking, less suitable, and therefore still less frequently used. An exception is the determination of copper and zinc in brass,[35] but this does not come within the scope of the present survey, in which (in addition to its limitation to light metals and alloys) emphasis is placed on applications of polarography that have an obvious advantage over other methods.

Although the survey deals with metals in the first place, much of it will apply to the testing of salts (chemicals) as well; for the metals are tested in the form of salt solutions during the polarographic procedure. This should be emphasised, as the polarograph does not seem to have found the wide application it deserves in the testing of chemicals for impurities.

1. THE ALKALI METALS

For general information about the determination of the various alkali metals themselves, see Heyrovsky's[7] or Kolthoff and Lingane's monographs[40]; as explained before, their determination only comes within the scope of this section if one of them should be a constituent or an impurity of a metal or alloy of which another one is the base. In view of the coincidence of the half-wave potentials of sodium, potassium, rubidium and caesium this only offers any promise with lithium metal or lithium based alloys, and even there the distance between the half-wave potentials is only about 0·15 v., rather smaller than is desirable.

The alkali metals have a very negative half-wave potential; they show little tendency to hydrolyse or to precipitate in the course of pH adjustment or on mixing with the usual ground solutions; the polarographer can therefore expect to have a free hand in obtaining satisfactory waves of a variety of components or impurities. Many of the difficulties encountered in the analysis of, say, aluminium and ferrous alloys, would not arise here at all.

In spite of these very favourable conditions, surprisingly little has been published on the subject; an old paper by Hohn[1] on impurities in lithium was all that could be found in the literature. As lithium has the most negative half-wave potential of all the alkali metals, it is possible to determine other alkali metals (or at least their sum) in presence of lithium, apart from all the nobler metals.

2. ALKALINE EARTH METALS

The remarks above about alkali metals—favourable conditions and yet little research work done—apply almost as much to the alkaline earth metals (except magnesium and magnesium alloys).

The reason obviously lies in the comparatively small practical importance of these metals, but again it should be pointed out that polarographic methods can be applied to salts, *i.e.*, to chemicals as well as to metals. It should be of some practical importance that under suitable conditions calcium, barium and strontium can be distinguished from each other polarographically. According to the sequence of their half-wave potentials,[2] a small content of calcium could not be determined polarographically (at least not without resorting to chemical separation) in a base of barium or strontium; but the determination of barium or strontium in calcium metal or salts should be nearly as easy as that of any of the "classical" objects of polarographic technique, say cadmium or zinc.

It may be worth while pointing this out, as alloys of barium and aluminium in a calcium base find practical application as "getters" in radio and vacuum technique; it appears that so far no use has been made of this possibility and a recent monograph about the metallurgy and technology of calcium* does not seem to contain the word "polarography" at all.

A paper on the determination of strontium as impurity in calcium carbonate has been published in Germany[3] during the war.

Further data are contained in Heyrovský's book.[4]

Before going over to those light metals that are usually thought of when this term is used, *i.e.*, magnesium, aluminium and beryllium, a few short remarks may be made about the rare earth metals.

3. RARE EARTH METALS

These metals are included in this survey for two reasons. One is sentimental, or historical: it is interesting that the only polarographic experiment that could be found about one of the most important elements of this group dates back to one of the very first papers in which the polarograph was described by Heyrovský and Shikata[37] and the word "polarography" was coined. One of the examples given there of the possible applications of the new technique is a beautiful wave of lead as impurity in cerium metal, and it becomes clear from this graph that here, as with the alkali and alkaline earth metals, practically the entire voltage range is available for the determination of whatever other elements may need to be determined. This similarity is the second reason for including the rare earth group in this survey, although only some of its members have a specific gravity smaller than that of zinc.

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* *Calcium, Metallurgy and Technology*, by C. L. Martell and C. Hardy, Reinhold Publishing Corporation, New York, 1945.

There is, however, one rare earth metal that has not a very negative reduction potential—europium, with a half-wave potential of only $-0{\cdot}77$ v.—a circumstance that has been utilised for determining this element in presence of others of the same group[38]; the same paper also deals with the determination of zinc in presence of rare earth metals other than europium.

4. MAGNESIUM

Magnesium and its alloys are of much greater practical importance than the metals previously referred to.

In Gull's well known method,[5] the metal is dissolved in hydrochloric acid and the pH is adjusted to the lowest acidity at which aluminium will remain unprecipitated, bromophenol blue being used as indicator.

Lead, cadmium, zinc and aluminium, if present in suitable proportions, can be determined in one operation, and as the existing commercial alloys fulfil this condition the method is very useful. It should even be possible to obtain at the same time a semi-quantitative determination of small quantities of copper and iron; but owing to the great ease with which these elements can be determined photometrically, this point has not been followed up.

The size of sample recommended by Gull (80 mg. per 200 ml. of final solution)—unless this figure is a misprint—seems rather small; it may be large enough for the aluminium in alloys containing 5 to 10 per cent. of this metal, but a concentration about 10 times greater (100 to 200 mg. per 25 ml. of final solution) seems preferable in order to obtain waves of suitable size for the other elements, particularly lead. This requires, of course, an adequate increase of the quantity of hydrochloric acid used.

With very high aluminium contents (15 per cent. and more) the use of the lowest sensitivity of the instrument and possibly a slight dilution of the solution may be necessary.

Gull's method would fail if the nobler metals were present in much larger quantity, and difficulties would also arise in presence of substantial amounts of tin or nickel, as the wave of bivalent tin coalesces with that of lead and the wave of nickel with that of zinc.

In commercial alloys these two elements are rarely present in alloying quantities (though Specification DTD350A does call for 4·5 to 7·5 per cent. of tin) and it is easy to test for their presence by well known methods; the photometric and volumetric methods in general use for determining nickel and tin in aluminium alloys can be applied without substantial modification to magnesium alloys.

With uncommon alloys or "freaks," encountered occasionally, particularly among swarfs and similar material, it is useful to apply the method published by the writer for the determination of lead in aluminium alloys.[33] The use of this procedure, which is a simplification of Kolthoff and Matsuyama's technique,[18] will overcome a number of possible difficulties: copper will be precipitated as the thiocyanate and iron will be reduced; tin will be oxidised to the quadrivalent form, in which it does not give a wave at all at the pH of the solutions; the waves of nickel and zinc are well separated in this medium. No interference from any of these elements is therefore to be expected in the determination of lead and zinc.

If this method[33] is to be applied to magnesium alloys, it is necessary to modify the procedure slightly in order to obtain the required pH. A short description of the two techniques is given below for greater clarity. (A) is essentially Gull's technique, with minor modifications. (B) is the writer's.

(A) *Copper, tin and nickel absent*—Attack a weighed sample, 100 to 200 mg., in a 25-ml. volumetric vessel (a Pyrex boiling tube, 6 in. by 1 in., carefully calibrated at 25 ml. and 40 ml., is very suitable), with 2 ml. of 5 *N* hydrochloric acid and a little water. Complete the reaction by immersing the vessel in hot water. Cool and add 0·1 ml. of 0·04 per cent. bromophenol blue solution containing 1·5 ml. of 0·1 *N* sodium hydroxide per 100 mg. of the dye. Then add, dropwise and with shaking, 1 *N* sodium carbonate* until the colour changes to green or blue. (The dye is bright yellow up to pH 2·8 and blue to violet beyond 4·6; green is the intermediate colour. As the solution is not strongly buffered if little aluminium is present, 1 drop of 1 *N* alkali easily makes the colour change from yellow to blue without any green becoming apparent.) Adjust the colour to green by adding, dropwise and with shaking, 0·1 *N* hydrochloric acid. Add water nearly to the 25-ml. mark. Allow to stand for several hours, to ensure that all the aluminium is in solution even if it has been temporarily precipitated, and observe the colour from time to time, adding some more indicator, if required, and adjusting to green, if necessary, by adding a drop or two of approximately 0·1 *N* sodium carbonate or hydrochloric acid. Add 0·5 ml. of 0·25 per cent. gelatin solution and make up to the mark. Mix and take a portion for the polarographic test, or, if a silver wire anode be used[39] (this is particularly advantageous in combination with the use of the above-mentioned calibrated boiling tubes), de-aerate and polarograph directly in the tube. This latter technique has the further advantage that, after the steps of lead and zinc have been recorded, the solution can very simply be adjusted to 40 ml. for further dilution should it be found too concentrated to give a suitable aluminium step with alloys containing 10 per cent. or more of this element. It is obvious that in this event a calibration must be made under the same conditions.

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* The author prefers sodium carbonate to caustic soda, recommended by Gull, as with the former it is easier to avoid temporary precipitation of aluminium.

(B) *Copper, iron, tin or nickel present in substantial quantity*—For determining lead and zinc in presence of the above metals, proceed as described for the determination of lead in aluminium alloys,[33] but before adding hydroxylamine and thiocyanate (for the purpose of reducing the iron and precipitating the copper), adjust the reaction as described in (A) above.

This procedure is, however, not suitable for the simultaneous determination of aluminium; although aluminium gives in this medium a wave *qualitatively* not inferior to that obtained by Gull's method, the results are low if the alloy contains substantial quantities of copper, probably owing to co-precipitation of aluminium with the copper at the low acidity necessary for precipitating the copper and reducing the iron by the combined action of hydroxylamine and potassium thiocyanate. In such circumstances a second sample has therefore to be processed by Gull's method, or the modification described above in (A), for the determination of aluminium; as the alloys in question practically always contain much more, or at least not less, aluminium than copper, iron, zinc, tin, lead or cadmium, none of these elements will cause any difficulty in the determination of the aluminium, although their waves precede that of aluminium. It is worth noting that the polarographic method is suitable only for comparatively large aluminium contents (say 1 per cent. and more) because of the hydrogen wave, which immediately precedes that of aluminium. As complex-formers inhibit the aluminium wave altogether and as lowering the acidity leads to hydrolysis (with deterioration of the wave), a compromise must be found, *viz.*, moderate acidity and high aluminium concentration. At the low galvanometer sensitivity required, the hydrogen wave does not then interfere. To process a second sample according to Gull's technique in such cases has a further advantage. Whilst in the thiocyanate medium the waves of nickel and zinc are separated, the nickel wave coalesces with that of cadmium; this is not so under Gull's conditions. The use of both techniques will therefore also contribute to clarify the situation if cadmium, nickel *and* zinc should all three be present; this, however, will be a very rare occurence.

Semerano and Ronchi[8] have severely criticised the reproducibility of results by Gull's method. The writer is disposed to think from his experience that their criticism is too severe.

Semerano and his school have published three different techniques for the polarographic analysis of magnesium.[8–10]

The first is very similar to that of Gull, except for the use of a different indicator: a mixture of methylene blue and methyl yellow, claimed to give a particularly sharp colour change, from violet to green, at pH 3·25.

The second method also begins by dissolving the alloy (0·1 g.) in hydrochloric acid but continues by separating the alloying elements from the bulk of the magnesium; this is effected by carefully neutralising to methyl red with dilute ammonia solution and then adding ammonium sulphide solution and centrifuging. The precipitate is washed by centrifuging, lithium chloride being added to the washing water to prevent any peptisation of aluminium, and the residue is dissolved in diluted hydrochloric acid (1 + 1). The solution is neutralised with lithium hydroxide, using the mixed indicator mentioned above, and made up to volume. After recording the wave of aluminium, methyl red is added and then some more lithium hydroxide, until the methyl red becomes yellow; the other components are then recorded, with adequate sensitivity, starting from zero potential. Owing to the very negative deposition potential of lithium, the diffusion current of the aluminium is very well defined under these conditions.

The third technique, dealing with impurities in pure magnesium metal, is not accurately described; it seems to consist in dissolving so much metal in hydrochloric acid that a practically saturated solution is obtained.

The work of Semerano and his colleagues has also been extended to the determination of traces of sodium in magnesium (further data about this have also been published by Heyrovský[6,7]) and further to the determination of antimony,[11] which seems to be a constituent of Italian magnesium alloys. In this method 0·5 g. of the alloy is dissolved in hydrochloric acid, with addition of a few drops of concentrated nitric acid, and then twice evaporated to dryness with addition of some hydrochloric acid, to drive off the nitric acid; the residue is taken up in Hohn's ground solution E (hydrochloric acid with gelatin) and antimony and lead are determined. An aliquot of this solution is then evaporated to dryness and the residue taken up in Hohn's ground solution C (ammoniacal ammonium chloride solution with tylose) for the determination of zinc and manganese. Aluminium and iron can eventually be determined by dissolving in hydrochloric acid the residue that remains undissolved in the ammoniacal ground solution C and neutralising with lithium hydroxide as described before.

The writer cannot claim experience with any of Semerano's methods.

The determination of copper, zinc and aluminium is also described in a report by Payne.[13]

5. ALUMINIUM

For practical purposes this is by far the most important of the light metals and it has accordingly been the subject of the greatest volume of published work, including a well known monograph,[29] and a comprehensive scheme,[18] which cannot be discussed here in detail for reasons of space.

In the writer's opinion the methods for zinc and lead deserve—at least at present—primary consideration, as for them the polarographic technique seems to offer greater advantage, in comparison with the other available "wet" methods, than it does in respect of the other elements (such as copper, iron and nickel) that also *can* be determined polarographically, but for which very rapid and good photometric methods are available.

A. *Determination of zinc*—Various methods have been proposed, many of which[18,19,29] were reviewed in the writer's papers[31,32] describing his own method.* About the latter method it may suffice to say that it has found large-scale routine application over a number of years and has proved to be reliable, rapid[34,36] and applicable to a very wide range of alloys.

Attention should be drawn to a method,† hitherto unpublished, by H. C. Davis,[28] from the Royal Aircraft Establishment, Farnborough. It begins with *aqua regia* attack, followed by neutralisation with ammonia in presence of citrate.

If much copper is present it is separated by addition of some sodium sulphide in presence of excess of 1 *N* sulphuric acid, followed by filtration. An aliquot of the filtrate is neutralised and excess of formic acid and again some sodium sulphide are added. In presence of only small amounts of copper, the first separation is unnecessary and the combined sulphides of copper and zinc are precipitated in the formic acid medium.

The sulphide precipitate is extracted with a measured quantity of 2·5 *N* sulphuric acid, excess of sulphide is destroyed by bromine, the excess of which is boiled off, and the solution is then poured into an excess of sodium hydroxide solution so adjusted that the final concentration becomes 0·1 *N*. After addition of a standard solution of lead and enough gelatin to produce a final concentration of 0·002 per cent. the solution is made up to the mark and the polarogram recorded. The lead addition serves as internal standard or "pilot-ion."

The "pros" and "cons" of this procedure cannot be discussed here in detail, but it should be pointed out that the separation from the bulk of the aluminium makes it technically easy to restrict the final alkalinity to 0·1 *N*, which produces very well defined graphs, as illustrated in Fig. 1.

The same principle of separation from the bulk of the metal, *i.e.*, from the aluminium, by means of sulphide, has been used by Semerano and Capitanio[24–26] for the determination of impurities in "super-pure" aluminium. In order to attain great sensitivity, these authors begin with a 5-g. sample. After an alkaline attack, using very pure potassium hydroxide and redistilled water, a small quantity of hydrogen sulphide is passed slowly into the alkaline solution and the precipitate is collected by centrifuging and washed with dilute sulphide solution. The residue is taken to dryness with nitric acid. From this residue copper, cadmium, nickel and zinc are extracted with 1 ml. of an ammoniacal solution, which is 0·5 *N* in ammonia, 0·1 *N* in ammonium carbonate and 1·5 *N* in ammonium chloride and contains 5 per cent. of sodium sulphite and 0·005 per cent. of gelatin. After centrifuging, the polarogram is recorded in a suitable cell. The elements mentioned above can all be recorded in one graph. The residue in the centrifuge tube is washed, dissolved in hydrochloric acid and taken to dryness. The residue is extracted with 1 ml. of a solution similar to Hohn's ground solution P (9 parts of saturated ammonium chloride solution, 10 parts of 2 per cent. tylose solution and sufficient hydrochloric acid to make the mixture 0·1 *N*) and then the waves of iron and lead can be recorded.

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* After this paper went to print a paper by B. A. Scott appeared (*Analyst*, 1948, **73**, 613). The method described, however, is applicable only if the alloy does not contain much more nickel than zinc, and therefore fails with many important alloys.

† Thanks are due to the Ministry of Supply and to Mr. Davis for permission to discuss the method here.

Owing to the size of the sample taken and the small volume of the final solution, as little as 0·00003 per cent. of the elements mentioned is claimed to be measurable. Possible contamination by the reagents must be allowed for.

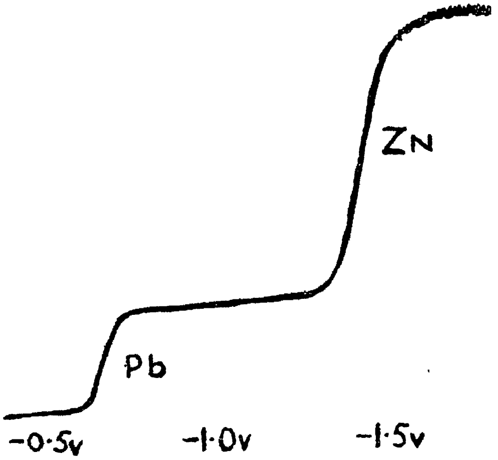

Fig. 1

Steps for lead and zinc in 0·1 *N* NaOH
Zn 0·02 g./litre; Pb 0·02 g./litre; gelatin 0·02 g./litre. Sensitivity 1/10
(From Report MET 19, by courtesy of the Ministry of Supply and Mr. H. C. Davis)

For the commercially pure aluminium the same authors propose a rapid method[23] similar to the well-known old method of Hohn,[36] but avoiding the excessive concentration used by Hohn, which leads to losses by adsorption on the undissolved aluminium hydroxide. Iron, copper and lead can be determined as well as zinc, and the lower limits claimed are 0·015 per cent. of iron, 0·006 per cent. of copper, 0·01 per cent. of lead and 0·015 per cent. of zinc. The method is not suitable for alloys.

The most recent paper is that by M. Spalenka,[20] which describes methods for determining manganese and copper as well as zinc. The procedure for zinc differs according to the composition of the alloy. (*a*) For the determination of copper in all alloys, and of zinc in alloys containing little copper and nickel, 0·3 g. of alloy is dissolved in hydrochloric acid (with addition of a little nitric acid or hydrogen peroxide to dissolve the copper); excess of ammonia is added and, after cooling, 1 ml. of saturated sodium sulphite solution and some gelatin are added and the solution is made up to 100 ml. (*b*) Alloys rich in copper and nickel are attacked with caustic soda (0·5 g. of alloy and 10 ml. of 3·5 *N* sodium hydroxide). When the reaction is complete, 10 ml. of water and, after cooling, 15 ml. of a solution containing 45 g. of ammonium chloride and 350 ml. of concentrated ammonium hydroxide solution per litre are added, followed by 1 ml. of saturated sodium sulphite solution and some gelatin. The solution is made up to 50 ml. and polarographed.

It is admitted by Spalenka that in procedure (*a*) the precipitated aluminium hydroxide adsorbs some copper and it is well known[29,31,32] (although not mentioned by Spalenka) that an alkaline attack does not extract the total zinc from an alloy containing considerable quantities of copper and other alkali-insoluble constituents. It is thought by Spalenka that these sources of error can be eliminated by calibrating under the same conditions, when an identical loss will occur. The writer thinks, however, that this is a dangerous principle, as it may be difficult to ensure that the same factors act each time to the same extent.

The addition of ammonium chloride - ammonia solution improves the shape of the zinc wave, compared with that obtained in pure sodium hydroxide solution.

For the determination of manganese, 0·5 g. of the metal is dissolved in hydrochloric acid and a few drops of hydrogen peroxide. Rochelle salt, sulphite and a sodium hydroxide solution containing potassium cyanide are added and, before the cyanide, some *p*-methylaminophenol sulphate (Metol), to prevent the manganese precipitating at alkaline reaction.

B. *Determination of lead*—The method of Kolthoff and Matsuyama[19] has been simplified by the writer.[33] This procedure is applicable to all usual types of alloys and is simple and reliable, whilst other procedures[19,22,23,24,27,29,30] are either slower or applicable only to a restricted range of alloys, mainly to pure aluminium.

C. *Determination of sodium*—Several methods have been proposed[14–17] which should be able to compete with existing "wet" methods; the writer has, however, no personal experience of them. The method of Urech and Sulzberger is based on separation from the bulk of the aluminium by saturating with hydrogen chloride gas.

6. Beryllium

Very little work has been published about the application of polarography to this metal and nothing, to the writer's knowledge, about polarographic determinations in beryllium-based alloys. It seems likely, in view of the general properties, that the methods developed for aluminium alloys can be applied to beryllium as well. Whether beryllium itself can be determined—in a similar manner to the determination of aluminium in magnesium alloys—is not clear, but is probably of no practical importance, as what would most likely be needed is differentiation between aluminium and beryllium, which does not appear to be practicable.

The permission of the Directors of International Alloys Ltd. to publish this survey is gratefully acknowledged.

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	Base metal	Element determined	
1.	Li	Pb, Al, Fe, Zn	Hohn, H., *Chemie*, 1935, **48**, 463.
2.	Ca	Ba, Sr	Zlotowski, I., and Kolthoff, I. M., *J. Amer. Chem. Soc.*, 1944, **66**, 1431.
3.	„	Sr	Straumanis, M., and Dravnieks, A., *Z. anal. Chem.*, 1940, **120**, 168.
4.	„	„	Novak, J. V. A., and Ulrich, E., in Heyrovský's *Polarographie*, 1941, p. 303.
5.	Mg	Zn, Pb, Mn, Al	Gull, H. C., *J. Soc. Chem. Ind.*, 1937, **56**, 177T.
6.	„	Na	Heyrovský, J., *Metallwirtschaft*, 1944, 333.
7.	„	„	——, *Polarographie*, Springer, Vienna, 1941, p. 295.*
8.	„	Zn, Mn, Al and others	Semerano, G., and Ronchi, I., *Atti R. Ist Venet. Scienze Lett. Arti.*, 1940–41, 100, II, 397.
9.	„	„ „ „	Semerano, G., and Capitanio, V., *Mikrochemie*, 1942, **30**, 71.
10.	„	„ „ „	Semerano, G., *Chemie*, 1942, **55**, 351.
11.	„	Pb, Sb	Volpi, A., *Ricerca Scientifica Ricostruzione*, 1947, **17**, No. 9.
12.	„	Al, Zn	Geller, B. A., and Zanko, A. M., *Zavod. Lab.*, 1939, **8**, 1030; 1940, **9**, 513; *Chem., Zentrblt.*, 1942, I, 1666.
13.	„	Cu, Zn, Al	Payne, S. T., Report No. 729 (restricted to members) of Brit. Non-Ferrous Metals Res. Assoc.
14.	Al	Na	Heyrovský, J., *Metallwirtschaft*, 1944, 333.
15.	„	„	Majer, V., *Z. anal. Chem.*, 1933, **92**, 321, 401.
16.	„	„	Urech, P., and Sulzberger, R., *Helv. Chim. Acta*, 1944, **27**, 1074.
17.	„	„	Weaver, J. R., and Lykken, L., *Anal. Chem.*, 1947, 372.
18.	„	Cu, Fe, Ni, Zn, Pb	Kolthoff, I. M., and Matsuyama, G., *Ind. Eng. Chem.*, *Anal. Ed.*, 1945, **17**, 615.
19.	„	Ni, Zn, Pb	Jablonski, F., and Moritz, H., *Aluminium*, 1943, **25**, 291; 1944, **26**, 38, 97, 245.
20.	„	Cu, Zn, Mn	Spálenka, M., *Z. anal. Chem.*, 1947, **128**, 42.
21.	„	Sb, Zn, Pb, Sn	Payne, S. T., see ref. 13 above.

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* Lithoprinted by Edwards Brothers Inc., Ann Arbor, Michigan, U.S.A., by authority of the Alien Property Custodian.

22.	"	Zn, Pb, Cd	Ginsberg, H., *Leichtmetallanalyse*, printed as a MS. in restricted numbers in Oslo, 1945, p. 236.
23.	Pure Al	Fe, Cu, Pb, Zn	Semerano, G., and Capitanio, V., *Atti R. Istit. Venet. Scienze Lett. Arti.*, 1941–42, **101**, II, 333.
24.	High pur. Al	Cu, Ni, Zn, Cd, Pb	Capitanio, V., *Ibid.*, 1940–41, **100**, II, 419.
25.			Semerano, G., and Capitanio, V., *Mikrochem.*, 1942, **30**, 71.
26.			Semerano, G., *Chemie*, 1942, **55**, 351.
27.	Al	Fe, Cu, Pb, Zn	Nickelson, A. S., *Analyst*, 1946, **71**, 58.
28.	"	Zn	Davis, H. C., Report Met. 19, Royal Aircraft Estab., Farnboro', Hants., 1947.
29.	"	Sb, Cu, Pb, Ni, Zn, Sn	Publication No. 401, The Brit. Aluminium Co. Ltd., 1946, London, E.C.2.
30.	"	Pb, Zn, Sb	Coates, A. C., and Smart, R., *J. Soc. Chem. Ind.*, 1941, **60**, 249.
31.	"	Zn	Stross, W., and Osborn, G. H., *Light Metals*, 1944, **7**, 323.
32.	"	"	Stross, W., *Metallurgia*, 1947, **36**, 163, 227.
33.	"	Pb	——, *Ibid.*, 1947, **37**, 49.
34.	"	Zn	——, *Industrial Chemist*, Sept., 1945.
35.	"	"	Hohn, H., *Chem. Analyse mit dem Polarographen*, Springer, Berlin, 1937.
36.	"	"	Phillips, D. F., *Metallurgia*, Dec., 1946, 113.
37.	Cerium	Pb	Heyrovský, J., *Rec. Trav. Chim. Pays-Bas*, 1925, **44**, 496.
38.	Rare earths	Eu, Zn	Holleck, L., *Z. anal. Chem.*, 1931, **116**, 161.
39.	—	—	Lingane, J. J., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 329.
40.	—	—	Kolthoff, I. M., and Lingane, J. J., *Polarography*, Interscience Publishers Inc., New York, 1941.

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Polarographic Determination of Thiomersalate

BY J. E. PAGE AND J. G. WALLER

IN view of the difficulty of measuring small amounts of thiomersalate (sodium ethyl mercurithiosalicylate) in pharmaceutical preparations, we have developed a polarographic method for its determination. Herasymenko, Heyrovský and Tančakivský,[1] and Kolthoff and Miller[2] showed that mercurous and mercuric ions yield well defined diffusion currents that are directly proportional to the concentration of mercurous or mercuric salt, but very little information has been published on the polarographic behaviour of mercury complexes. Recently, Poma[3] described the behaviour of mercury solutions containing sodium perchlorate as the indifferent electrolyte and Malyugina, Shchennikova and Korshunov[4] and Shchennikova and Korshunov[5] have studied mercuric iodide complexes.

TABLE I

EFFECT OF HYDROGEN ION CONCENTRATION ON POLAROGRAMS GIVEN BY 0·02 PER CENT. THIOMERSALATE SOLUTIONS

Indifferent electrolyte	pH value	First step		Second step	
		Half-wave potential, v.	Step height in microamp.	Half-wave potential, v.	Step height in microamp.
5·0 *N* Hydrochloric acid ..	—	—	0	−0·50	1·25
1·0 *N* " ..	—	—	0	−0·48	1·35
0·1 *N* " ..	0·9	−0·10	1·15	−0·46	1·30
0·01 *N* " ..	1·7	−0·10	0·75	−0·53	1·00
0·025 *M* Potassium hydrogen phthalate	4·0	−0·10	0·75	−0·63	1·20
0·025 *M* Sodium phosphate mixture	7·0	−0·10	0·70	−0·80	0·95
0·025 *M* Sodium borate ..	9·2	−0·16	1·55	−0·95	0·95
0·1 *N* Sodium hydroxide ..	12·0	−0·31	0·55	−0·82	0·50

We found that thiomersalate in either acid or neutral solution gave a characteristics polarogram with two well defined steps (Fig. 1). The position and height of the steps formed by a 0·02 per cent. solution of thiomersalate varied considerably with change in hydrogen ion concentration and chemical composition of the solution (Table I). The first step was not formed in strongly acid solutions: the second step was the more suitable for the determination of thiomersalate.

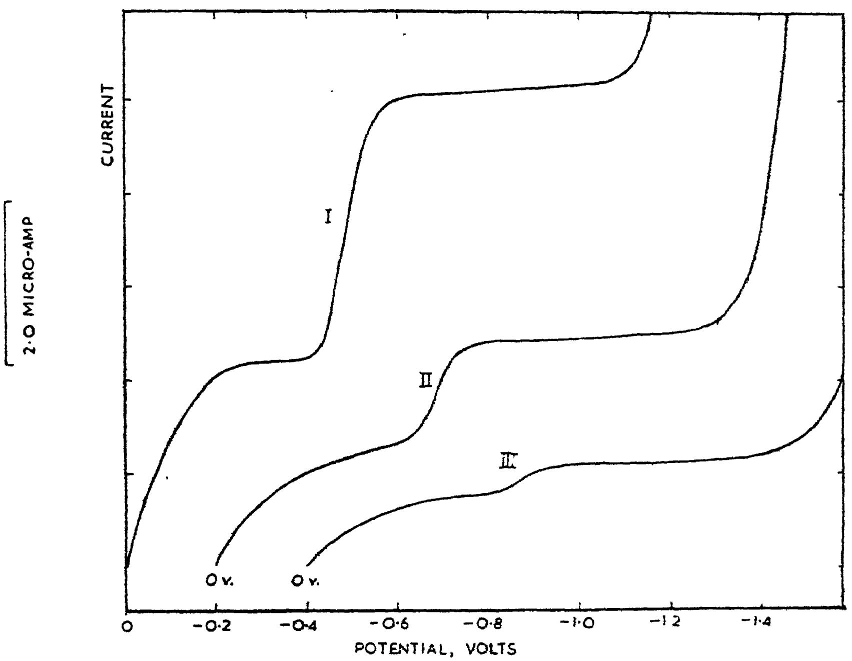

Fig. 1. Polarograms for thiomersalate in 1·0 *N* hydrochloric acid containing 0·01 per cent. of gelatin: curve I 0·05 per cent.; curve II 0·02 per cent.; curve III 0·005 per cent.

Thiomersalate in 0·1 *N* and 1·0 *N* nitric and perchloric acids gave similar steps to those produced in the same concentrations of hydrochloric acid.

Experimental

A Cambridge recording polarograph calibrated to read in microamps. was used in this investigation. All potentials were measured directly against the saturated calomel electrode. The dropping mercury capillary had the following characteristics. At a pressure of 55·5 cm. of mercury the drop-time (t) on open circuit in 0·1 *N* potassium chloride at 25° C. was 3·13 sec., the weight of mercury dropping per sec. (m) was 1·82 mg., and $m^{2/3} t^{1/6}$ was 1·80.

Our recommended procedure for the determination of thiomersalate in a solution is as follows.

Add 1·0 ml. of concentrated hydrochloric acid and 1·0 ml. of 0·1 per cent. gelatin solution to a volume of solution containing between 0·1 and 1·0 mg. of thiomersalate and dilute the mixture to 10 ml. Transfer a 3-ml. portion of the diluted solution to the polarograph cell, bubble nitrogen through it for 10 minutes to remove oxygen and examine it over the potential range 0 to −0·8 v. Measure the height of the step appearing at about −0·5 v.

For solutions containing suspended matter (*e.g.*, alum-precipitated vaccines), either the precipitate was filtered off before addition of acid or the final solution containing suspended matter was examined in a polarograph cell connected through an agar bridge to a saturated calomel electrode, the latter being used as anode. Determinations by the two procedures on vaccines containing 0·01 per cent. of thiomersalate agreed to within 10 per cent.

Some typical polarograms for thiomersalate in acid solution are shown in Fig. 1. The results set out in Table II show that the diffusion current of the second step formed in 1·0 *N* hydrochloric acid is proportional to concentration over the range 0·002 to 0·05 per cent. of thiomersalate.

TABLE II

RELATION BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF THIOMERSALATE IN 1·0 *N* HYDROCHLORIC ACID

Percentage of thiomersalate C	Diffusion current in microamp. *i*	*i*/C
0·002	0·15	75
0·005	0·30	60
0·010	0·65	65
0·020	1·30	65
0·050	3·50	70

Experiments on the recovery of added thiomersalate from pharmaceutical preparations are summarised in Table III. The recoveries from vaccine preparations are very good, but those from a crude prolactin preparation and from liver extract preparations are less satisfactory. For vaccines containing about 0·01 per cent. of thiomersalate the recovery was 90 per cent. or more.

TABLE III

RECOVERY OF THIOMERSALATE FROM PHARMACEUTICAL PREPARATIONS

Preparation	Thiomersalate added to 100 ml. of preparation mg.	Thiomersalate found in 100 ml. of preparation mg.	Recovery of thiomersalate %
Whooping cough vaccine	2·0	1·8	90
" "	10·0	9·5	95
" "	50·0	45·0	90
" "	100·0	95·0	95
Prolactin preparation ..	10·0	8·5	85
" " ..	15·0	13·5	90
Liver extract	10·0	8·0	80

Antiseptics of the phenol type, such as *p*-chloro-*m*-cresol, most organic substances and metallic ions (except those of antimony, arsenic, bismuth, cadmium, tin, titanium and vanadium) do not interfere with the polarographic procedure for the determination of thiomersalate. Thus thiomersalate can be determined in vaccine preparations, but not in whole blood or in ter- and quinquevalent antimony preparations, such as stibophen and sodium antimony^{V} gluconate. Determinations on liver extracts containing about 0·01 per cent. of thiomersalate were usually about 20 per cent. low. Since this loss is consistent, a calibration curve can be constructed to allow for it.

BEHAVIOUR OF THIOMERSALATE IN AMMONIACAL COBALT BUFFER SOLUTIONS—

In view of the interest attaching to the catalytic steps formed by sulphydryl-containing amino acids (such as cysteine) in ammoniacal cobalt buffer solutions,[6] we have studied the behaviour of thiomersalate in similar solutions.

Ten ml. of a 0·01 per cent. thiomersalate solution were added to a mixture of 2·0 ml. of 1·0 *N* ammonium chloride, 2·0 ml. of 0·02 *M* cobalt chloride and 2·0 ml. of 1·0 *N* ammonium hydroxide, the solution was made up to 20 ml. with distilled water and an aliquot portion was polarographed over the potential range −0·8 to −2·0 v.

The polarograms obtained in this way showed steps at about −1·5 v.; these were surmounted by catalytic maxima at about −1·9 v. Although 0·01 per cent. of gelatin suppressed the maximum on top of the cobalt step at −1·1 v., it did not affect the catalytic maximum due to the thiomersalate. These catalytic steps were somewhat similar in shape to those formed by cysteine under the same conditions; they are illustrated in Fig. 2.

For solutions containing more than 0·001 per cent. of thiomersalate, the height of the catalytic step was roughly proportional to concentration and was about one-fiftieth that of the corresponding step formed by a cysteine solution of the same molar concentration. During the contact with ammoniacal cobalt buffer solution the height of the catalytic step increased slightly, whereas such contact tends to reduce the height of the step formed by cysteine. Although the catalytic step formed by thiomersalate is not likely to be of practical importance, it is of considerable theoretical interest.

POLAROGRAPHIC BEHAVIOUR OF OTHER MERCURY PREPARATIONS—

A number of mercury preparations were examined in acid solution at negative potentials (Table IV); of those tested only phenylmercuric acetate and nitrate formed distinctive steps (Fig. 3), the second being the more satisfactory. Unfortunately, owing to the low solubility of these salts in acid solution, it was not possible to study them at concentrations greater than 0·01 per cent. Mercuric and mercurous nitrate in 0·1 *N* nitric acid yield reduction waves at about +0·42 v. (Kolthoff and Miller[2]) and not at negative potentials.

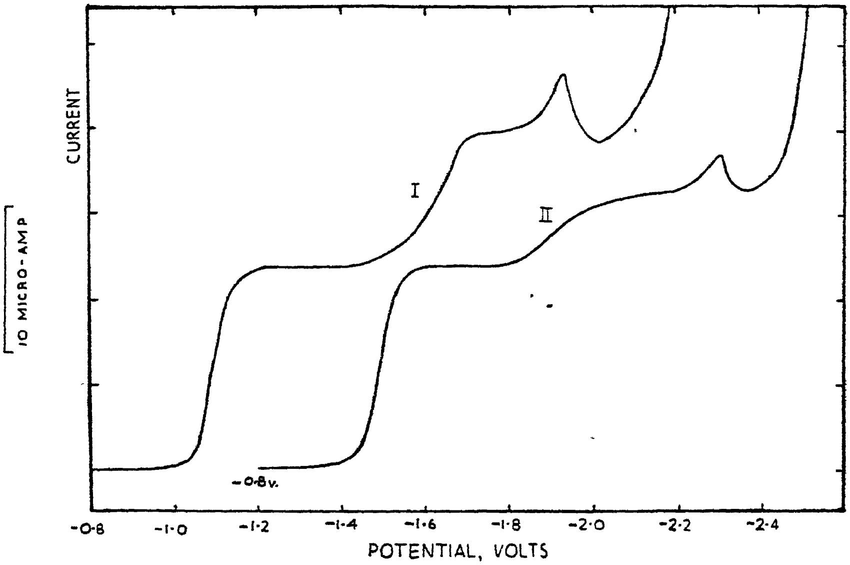

Fig. 2. Polarograms for thiomersalate in ammoniacal cobalt buffer solution containing 0·01 per cent. of gelatin: curve I 0·01 per cent.; curve II 0·005 per cent.

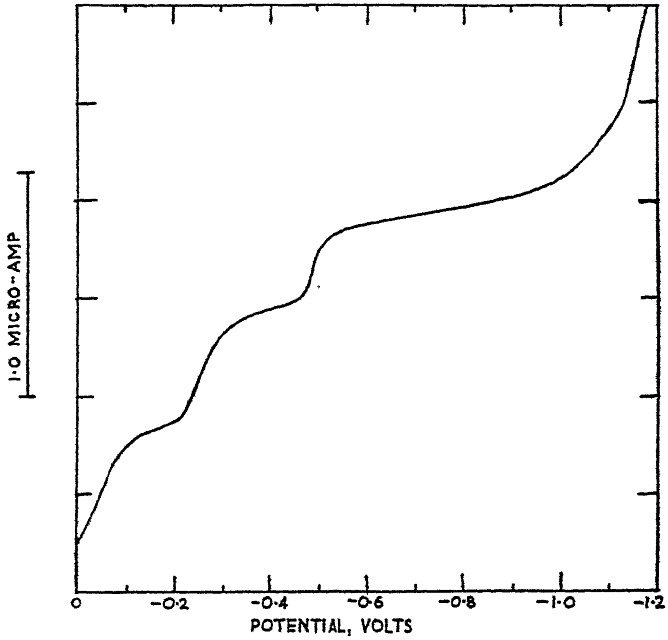

Fig. 3. Polarogram for $1{\cdot}0 \times 10^{-4}$ phenylmercuric nitrate in 1·0 *N* hydrochloric acid.

The steps shown by thiomersalate and phenylmercuric salts are probably formed by non-ionising mercury complexes and not by free mercury ions.

TABLE IV

POLAROGRAMS OF OTHER MERCURY COMPOUNDS

			First step		Second step	
Mercury preparation	Concn. of mercury preparation %	Indifferent electrolyte	Half-wave potential, v.	Diffusion current, microamp.	Half-wave potential, v.	Diffusion current, microamp.
Phenylmercuric nitrate	0·010	1·0 M HNO_3	−0·08	0·2	−0·62	0·6
"	0·004	1·0 M HCl	−0·25	0·3	−0·47	0·2
"	0·021	Sodium phosphate 0·025M (pH 7·0)	−0·12	0·5	−1·15	0·6
Phenylmercuric acetate	0·0035	1·0 M HCl	−0·25	0·4	−0·50	0·2
Mercurochrome ..	0·01	1·0 M HCl	—	0	—	0
Mercuric nitrate ..	0·01	1·0 M HNO_3	—	0	—	0
Mercuric chloride ..	0·01	1·0 M HCl	—	0	—	0

SUMMARY

In 1·0 N hydrochloric acid solution, thiomersalate gives a well defined step at —0·48 v. *versus* the saturated calomel electrode; this can be used for the determination of the antiseptic in vaccines and pharmaceutical preparations. Phenylmercuric acetate and nitrate, but not mercuric nitrate and chloride, form similar steps. In an ammoniacal cobalt buffer solution, thiomersalate gives catalytic steps of similar shape to, but of a much lower height than, those given by cysteine.

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Estimation of Aluminium in Beer

BY J. W. TULLO, W. J. STRINGER AND G. A. F. HARRISON

ALUMINIUM is usually present in beer in minute quantities and its accurate estimation is a matter of considerable difficulty. Much research has been carried out in this Laboratory on colorimetric methods employing aurine tricarboxylic acid, but no method involving this reagent was found to give accurate and reproducible results for beer. The most difficult part of the method is the separation of iron from aluminium.

The method of Gentry and Sherrington[1] for the "Direct Photometric Determination of Aluminium with 8-Hydroxyquinoline" was tried without success. Iron, and possibly some other elements, caused an interference which could not be completely eliminated. Gentry and Sherrington, however, recorded the fact that the chloroform solution of aluminium 8-hydroxyquinolinate "gave an intense greenish-yellow fluorescence on illumination with ultra-violet light," and suggested that this "might form the basis of a fluorimetric method for the determination of aluminium."

Following this suggestion a fluorimetric method has now been developed which is remarkable for its simplicity. The method would also appear to be highly specific for aluminium.

METHOD

OXIDATION OF SAMPLE—

Twenty-five ml. of beer are evaporated to a syrup in a silica Kjeldahl flask. Nitric acid is added and the flask gently warmed. About 4 ml. of sulphuric acid are then added and the flask is more strongly heated until signs of charring are noted. Nitric acid is added drop by drop to clear the dark colour, and this procedure continued until the sample will no longer char. The flask is then heated until sulphuric acid fumes are evolved, when about 1 ml. of nitric acid is added. When all the brown fumes have disappeared the sulphuric acid residue should appear colourless on cooling. About 0·5 g. of ammonium sulphate is added and washed in with a little distilled water, and the contents of the flask are again heated to fuming for about 5 minutes. This method, due to Pelouze,[2] is to ensure the complete removal of nitric acid.

(The nitric and sulphuric acids used were obtained by distillation of the "pure" acids from Pyrex glass and silica stills respectively.)

The time taken for oxidation is about 20 minutes. Ashing beer at 500° to 550° C. in a muffle furnace required a much longer time and resulted in the loss of about 20 per cent. of the aluminium present.

EXTRACTION OF ALUMINIUM—

No fixed concentration of 8-hydroxyquinoline in chloroform can be recommended for all circumstances, but it was found that 10 ml. of a 0·2 per cent. solution w/v always gave complete extraction of aluminium from 25 ml. of oxidised beer. Ten ml. of a 0·1 per cent. solution gave complete extraction for some beers, but when the aluminium or iron content of the beer was high (*i.e.*, more than about 3 parts per million), aluminium was not completely extracted. The procedure finally adopted for the extraction of aluminium from oxidised beer is as follows.

The sulphuric acid residue from the oxidation of 25 ml. of beer is diluted with about 40 ml. of distilled water, cooled and transferred with washing to a 100-ml. or 150-ml. separating funnel. To this, 10 ml. of a 0·2 per cent. solution of 8-hydroxyquinoline in chloroform are added. After a few seconds shaking, ammonia is added to adjust the pH to between 8 and 11. The separating funnel is then vigorously shaken for about 30 seconds, and the chloroform layer on settling is run off into a 20-ml. graduated flask. The aqueous layer is extracted with two 4-ml. portions of chloroform, which are added as washings to the flask, and the volume adjusted to 20 ml. with chloroform.

The extract is poured into a small Erlenmeyer flask and clarified by addition of about 1 g. of anhydrous sodium sulphate. This extract, which contains all the aluminium as the 8-hydroxyquinolinate, and also the 8-hydroxyquinolinates of iron and other elements, is then diluted 4-, 10- or 20-fold with chloroform (*i.e.*, 5, 2, or 1 ml. made up to 20 ml.).

If the undiluted extract is illuminated with ultra-violet light in the fluorimeter, a relatively intense fluorescence appears at the bottom of the cell, progressively weakening towards the top of the cell, owing to absorption of ultra-violet light. A chloroform solution of 8-hydroxyquinoline of concentration greater than about 0·05 per cent. w/v appreciably absorbs ultra-violet light. This effect is eliminated when the extract is diluted to 4 times its volume with chloroform. Similarly, if the extract is dark coloured (owing to the presence of iron or other 8-hydroxyquinolinates), dilutions of 10- or 20-fold may be employed to reduce the colour to a point at which no interference is caused.

MEASUREMENT OF INTENSITY OF FLUORESCENCE—

The diluted extract as obtained above is exposed to ultra-violet light (filtered through Wood's glass) in the Spekker fluorimeter, a Hilger No. 5 (green) filter being placed in front of the measuring photo-cell. With the chloroform solution in position and the drum set at zero the photo-cells are balanced. A solution containing 200 μg. of fluorescein per 150 ml. of distilled water is next placed in position, and the drum rotated to obtain a balance. The drum reading R is then noted and the value of the antilog of $(2 - R)$ is calculated. From this value the amount of aluminium in the extract may be found by reference to the appropriate standard curve.

This method of using the Spekker fluorimeter has been found very convenient. The drum scale of the instrument is logarithmic, and is such that the reading $R = \log a$, where $1/a$ is the fraction of the full aperture. The graph of $1/a$ against the concentration of fluorescent substances is approximately linear for dilute solutions. It is more convenient to graph 100/*a*, *i.e.*, antilog (2 — R), whereby small decimal quantities are avoided.

STANDARD CURVES—

Standard curves are made by extracting 0 to 80 µg. of aluminium in solution under the same conditions as in the test (*i.e.*, ammonium sulphate - ammonia solution, pH 8 to 11) with 10 ml. of a 0·2 per cent. solution of 8-hydroxyquinoline in chloroform. The extracts are adjusted to 20 ml. volume, and diluted 4-, 10- and 20-fold with chloroform, and the intensity of fluorescence of these diluted solutions measured in the fluorimeter against a fluorescein standard as described above. The results obtained are shown in Table I.

TABLE I

FLUORESCENCE OF STANDARD ALUMINIUM SOLUTIONS

µg. Aluminium per 20 ml. of undiluted extract	Dilution × 4		Dilution × 10		Dilution × 20	
	Drum reading R*	$\frac{100}{a}$	Drum reading R*	$\frac{100}{a}$	Drum reading R*	$\frac{100}{a}$
0	1·55	2·8	1·75	1·8	2·0	1·0
5	0·54	28·8	—	—	—	—
10	0·27	53·7	0·57	26·9	0·815	15·3
15	0·135	73·3	—	—	—	—
20	0·033	92·6	0·30	50·1	0·56	27·5
30			0·15	70·8	0·38	41·7
40			0·05	89·1	0·28	52·5
50					0·19	64·6
60					0·12	75·9
70					0·068	86·5
80					0·015	96·7

* R = log *a*.

INTERFERENCE—

Iron and other elements giving coloured 8-hydroxyquinolinates extractable by chloroform at pH 8 to 11 may interfere by absorbing the ultra-violet or fluorescent light, or both.

The removal of iron from solution by adjusting the pH to 2·0 and extracting with an 8-hydroxyquinoline solution in chloroform, as suggested by Gentry and Sherrington,[1] was found to be satisfactory for solutions containing only calcium, magnesium, potassium, ammonium, chloride, sulphate and phosphate ions. Iron was not, however, completely removed from oxidised beer by this method.

For the fluorimetric method it has not so far been found necessary to remove iron or other elements from any samples of beer examined. The ratio of iron to aluminium present may exceed 10 to 1 without causing interference. The sensitivity of the method is sufficiently great to permit of diluting the fluorescent solution to an extent that eliminates interference by iron or other metals giving coloured 8-hydroxyquinolinate soluble in chloroform. The requisite dilution still gives an intensity of fluorescence measurable by the Spekker fluorimeter.

SPECIFICITY—

In testing the method for specificity, solutions containing 50 µg. of different metals in about 100 ml. of an aqueous ammonium sulphate - ammonia solution of pH 8 to 11, were extracted with a 0·2 per cent. chloroform solution of 8-hydroxyquinoline as in the test, and the extracts examined in ultra-violet light. None of the following metals gave a fluorescent extract under these conditions: bismuth, calcium, cerium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, silver, thallium, thorium, tin, tungsten, uranium, vanadium.

With cadmium the extract exhibited a faint yellow fluorescence, which diminished in intensity as a precipitate formed. The extract from a zinc solution showed a faint greenish-yellow fluorescence, which also diminished in intensity with the formation of a precipitate. The fluorescence given by each of these is readily distinguishable from that of the aluminium 8-hydroxyquinolinate. If measured in the fluorimeter as being aluminium, 50 µg. of either metal would correspond to about 2 µg. of aluminium.

The extract from a solution of 50 µg. of beryllium (as beryllium sulphate) showed a similar fluorescence to that given by aluminium, and on measurement in the fluorimeter corresponded to the presence of 8·6 μg. of aluminium. The beryllium sulphate was then treated according to the method of Haber and Van Oordt[3] and a purified specimen of the acetate, $BeO.3Be(C_2H_3O_2)_2$, prepared. From this a further solution containing 50 μg. of beryllium was made up, which on extraction in the usual manner gave a faint greenish-yellow fluorescence, corresponding in the fluorimeter to 2·0 μg. of aluminium.

After a second purification the fluorescence was reduced to an extent corresponding to the presence of 0·4 μg. of aluminium in 50 μg. of beryllium. It would therefore appear that beryllium itself does not yield a fluorescent 8-hydroxyquinolinate, the fluorescence obtained here being due to aluminium present as an impurity.

ALUMINIUM IN BEER—

The method described has been applied successfully to the estimation of aluminium in beer. For beer of normal iron content (up to 1 part Fe per million), 4- or 10-fold dilution of the chloroform extract has been found to eliminate all interference, but for beer of high iron content a 20-fold dilution may be necessary. Aluminium added to beer was completely recovered. Table II shows four examples of recoveries obtained.

TABLE II

RECOVERY OF ALUMINIUM ADDED TO BEER

Al in 25 ml. beer μg.	Al added μg.	Total Al found μg.	Added Al recovered μg.	Recovery %
4·3	20	25·0	20·7	104
51·0	30	80·0	29·0	97
9·4	16	25·6	16·2	101
4·3	20	23·0	18·7	94

Duplicate analyses gave good agreement with one another. Seven examples of this are shown in Table III.

TABLE III

DUPLICATE ANALYSIS OF BEER SAMPLES

	μg. Al in 25 ml. beer		Al parts per million	
Sample No.	Test 1	Test 2	Test 1	Test 2
1	1·9	1·7	0·08	0·07
2	1·6	1·5	0·06	0·06
3	1·8	2·1	0·07	0·08
4	2·7	2·4	0·11	0·10
5	1·2	1·3	0·05	0·05
6	1·3	1·4	0·05	0·06
7	0·6	1·1	0·02	0·04

The method is capable of measuring as little as 0·01 parts per million of aluminium in beer.

SUMMARY

A simple and specific method for the fluorimetric estimation of traces of aluminium as the 8-hydroxyquinolinate has been described. Good recoveries have been obtained for aluminium added to beer, and duplicate analyses have agreed well. The method is very sensitive, and the presence of iron does not cause any interference.

The Directors of Messrs. Arthur Guinness Son & Co. Ltd. have kindly granted permission for the publication of this work.

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A Modification of the Micro-Analytical Test for Purity in Food for use in the Examination of Dried Fruits

By R. C. A. BRADSHAW and J. B. M. COPPOCK

In a previous paper describing the micro-analytical test for purity in food,[1] it was pointed out that the process of acid hydrolysis followed by pancreatin hydrolysis was not readily applicable to dried fruits. Moreover, a survey of the American literature on the subject has failed to reveal a suitable technique for dealing with such materials.[2] Past experience has shown that difficulties inherent in the hydrolytic method result in the filter paper upon which the extraneous materials are ultimately collected being deeply covered with fragments of skin from the fruit. These fragments have to be explored with a probe to allow adequate microscopic examination, with the result that an accurate determination of the rodent and other filth present becomes difficult or even impossible.

The prospect of applying this procedure to the extent likely to result from increasing analytical control of extraneous materials in foodstuffs was by no means attractive, and a method was evolved, therefore, using air and water agitation, by which the dried fruit could be vigorously scrubbed free from extraneous materials and these subsequently collected at an aviation spirit - water interface. This system was chosen because it was found that emulsions were formed if the scrubbing was carried out in the presence of both water and aviation spirit. Moreover, in such emulsions fleshy particles from the fruit were found to be present and these apparently contained a stabilising agent, which made the emulsion extremely difficult to break.

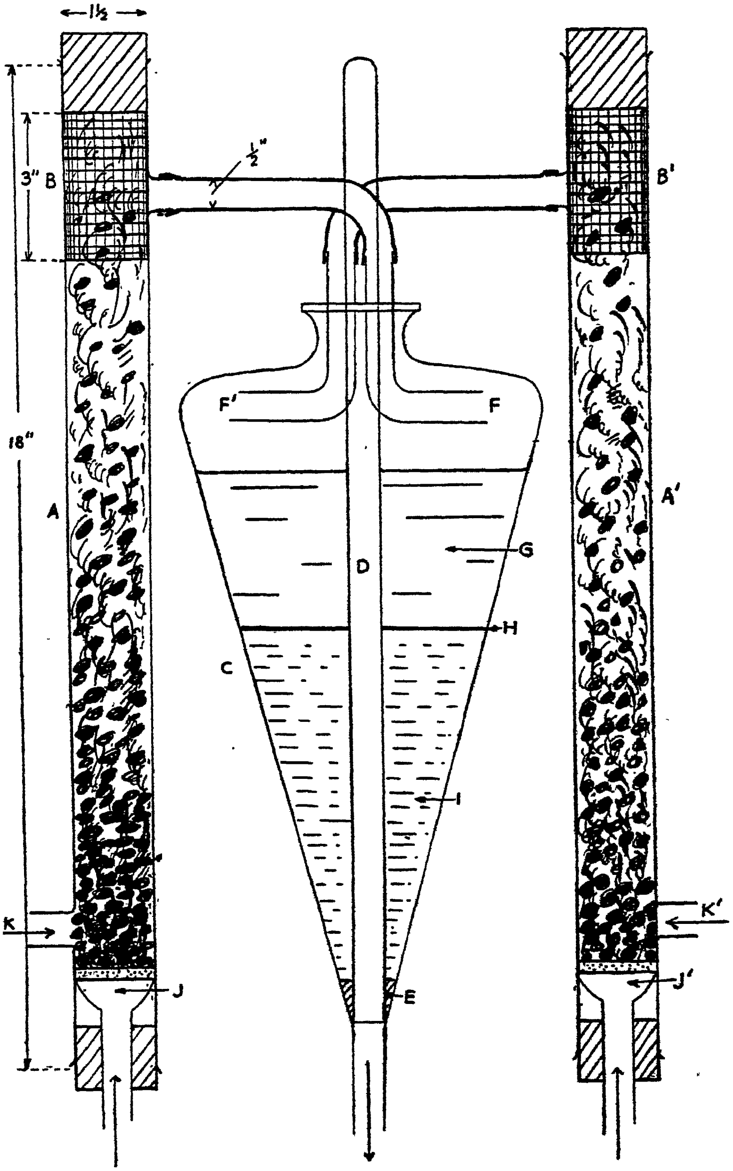
METHOD

The apparatus developed for the removal of the extraneous materials from fruit is illustrated in Fig. 1. In a convenient form of construction, slightly modified Liebig condenser jackets, A and A′, are fitted at one end with gauzes, B and B′ (mesh 10 per inch), so that large fleshy particles cannot enter and choke the upper side tubes. At the lower ends of the main tubes are fitted sintered filter-sticks, J and J′, of equal porosity and of the coarsest pore size available; these are connected to an air supply. By means of rubber tubes the upper side tubes are connected to glass angle tubes, F and F′, which deliver into a 2-litre separating funnel, C. The outlet of this is closed by a glass rod, D, fitted with a rubber seating, E. The separating funnel contains 800 ml. of aviation spirit, G, and, initially, 900 ml. of water, I, although this latter quantity may fall slightly in the course of the experiment.

The method of operation is to place 50 g. of the dried fruit in each vertical column. The side tubes, K and K′, are connected to the water supply and the water is allowed to fill approximately half of each column. It is important that the water be fed equally into the two columns; this may be accomplished by a suitable arrangement of rubber tubes and clips. The delivery of air to the filter-sticks is then begun. Equal amounts of air should be delivered to both columns; this may be judged by the relative bubbling that occurs. The amount of air required for the two columns is about 6 litres per minute at a pressure of 8 cm. of mercury. By suitable adjustment of the flow of air and water thorough agitation of the dried fruit may be produced and a frequent, though intermittent, delivery of water into C may be obtained.

This intermittent delivery is regarded as necessary as it has a flushing action and clears the delivery system. The glass angles, F and F′, are bent to accentuate the flushing action and also to direct the force of the jet at the moment of delivery on to the walls of the separating funnel. The water containing the extraneous material then slowly settles through the aviation spirit and the filth is held at the interface. If the rod, D, is eased, the water will slowly drain past E, so that the aviation spirit - water interface, H, remains approximately at the same level. The scrubbing of the fruit is continued for 30 minutes, unless the water in the column still remains cloudy after that time, in which event it is continued until the water clears. The supply of air is then stopped and the whole apparatus flushed with water for 10 minutes. The glass angle tubes are removed and most of the water is allowed to flow away. The small amount of the residual water and the whole of the aviation spirit is then poured on to a filter paper in the usual way and the filter paper is examined under a microscope.

Although in the illustration the water inlets, K and K′, are shown above the sintered stick, it is often more convenient to raise these sticks so that the water enters below the surface of the sintered discs. If this is done care should be taken to ensure that there is a sufficient annular space between the sintered disc and the condenser jacket to allow free passage for the water.



RESULTS

Before this new technique was adopted as routine a comparison was made with the conventional hydrolytic process. The results obtained are shown in Table I, which indicates the reliability and increased efficiency of the new technique. Several qualities of dried fruit from different sources were then examined by the method here described and some typical results are given in Table II. It is possible that, besides being applicable to dried fruit, this method may also be used for the removal of extraneous materials from whole wheat, nuts and the fruit in baked products. These possibilities are at present under examination.

TABLE I

COMPARISON OF METHODS

A = Water and air scrubbing method
B = Acid - pancreatin method

100-g. samples used in all experiments

Sample No.	Description of fruit	Method	Rodent hairs (RH)		Other animal hairs (AH)		RH + AH		Insect fragments	Bird fluff	
			No.	Length mm.	No.	Length mm.	Total No.	Total length mm.		No.	Length mm.
(i)	Sultanas	A	6	11·5	2	4·0	8	15·5	0	1	2·0
(i)	„	B	4	1·5	3	1·8	7	3·3	1	5	7·9
(i)	„	A	8	9·7	1	1·0	9	10·7	0	3	4·5
(i)	„	B	4	1·5	3	1·8	7	3·3	1	5	7·9
(ii)	Currants	A	1	15·2	—	—	1	15·2	10		
(ii)	„	B	1	1·7	—	—	1	1·7	1		
(iii)	„	A	1	0·1	—	—	1	0·1	1		
(iii)	„	B	—	—			—	—	2		

TABLE II

FILTH ESTIMATIONS ON CURRANTS AND SULTANAS

Method—Continuous scrubbing with air and water

All 100-g. samples

Sample No.	Description of fruit	Rodent hairs		Animal hairs		Insect fragments	Bird fluff		Total of all hairs	
		No.	Length mm.	No.	Length mm.		No.	Length mm.	Total No.	Total length mm.
1	Sultanas	4	5·4	2	2·8	0	1	0·4	6	8·2
2		4	2·0	1	1·0	0	3	1·8	5	3·0
3		6	11·5	2	4·0	0	5	7·9	8	15·5
4		3	5·6	1	0·5	1	2	2·3	4	6·1
5		3	1·3	3	4·3	1	4	1·6	6	5·6
6		2	0·3	2	0·8	1	5	5·5	4	1·1
7	„	1	4·5	3	35·9	0	3	4·7	4	40·4
8	„	2	1·7	4	10·1	0	8	8·3	6	11·8
		2	2·0	3	5·0	1	1	3·0	5	7·0
10	Currants	3	4·9	1	0·4	1	1	1·1	4	5·3
11		2	2·9	0	0	0	1	1·1	2	2·9
12		2	2·2	1	1·3	0	1	2·0	3	3·5
13		1	1·3	1	1·0	7	1	1·1	2	2·3
14		1	0·1	0	0	2	1	0·5	1	0·1
15		0	0	1	1·4	11	0	0	1	1·4
16		1	15·2	0	0	10	0	0	1	15·2
17		2	23·8	1	1·2	8	0	0	3	25·0

SUMMARY

A method is described for the removal of filth from dried fruits by air and water scrubbing. A comparison with the existing method is made and data are recorded indicating the amount of contamination with extraneous materials likely to be encountered in the examination of present-day dried fruits.

Thanks are due to the Council of the Association and the Department of Scientific and Industrial Research for permission to publish this work.

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THE BRITISH BAKING INDUSTRIES RESEARCH ASSOCIATION
CHORLEYWOOD LODGE
CHORLEYWOOD, HERTS.

October, 1948

The Determination of Aneurine in Pharmaceutical Products

BY RITA PATRICK AND J. F. H. WRIGHT*

THE assay of aneurine in pharmaceutical products by the thiochrome method of Jansen is lengthy and the precision is not high.[1,2] In a method for the estimation of aneurine in oats, Holman[3] substituted mercuric oxide for potassium ferricyanide as oxidising agent, the measurement of fluorescence being made in aqueous acetone solution. The application of this method to pharmaceutical products has been investigated and a modification having several advantages over the Jansen method has been developed. The technique is simpler and the precision and accuracy are higher. All reagents are stable for long periods and the tedious purification of *iso*-butanol is eliminated. The acetone used is prepared by merely heating the commercial product with activated carbon under reflux and distilling.

This method has been applied to routine analyses, the products including aneurine tablets, vitamin "B group" tablets, injectable "B group" solutions, a multi-vitamin concentrate in aqueous alcohol and chocolate malt based granules containing vitamins A, B_1, C and D and vanillin. For the last product a method for the separation of aneurine from interfering substances by adsorption on synthetic zeolite, based on that of Hochberg, Melnick and Oser,[4] has been developed.

EXPERIMENTAL

In the earlier part of this investigation fluorescence was measured by means of a Hilger Spekker fluorimeter; this was later replaced by a Klett instrument (Model 2070). Most of the results reported are based on measurements made with the latter, using a Corning No. 586 filter in the exciting beam and Corning Nos. 430 and 038 filters in front of the photo-cell. An aqueous solution of 1-methyl-5-aminoacridine hydrochloride was preferred to quinine sulphate as standard, because of its greater stability. Fluorimeter readings were found to be directly proportional to aneurine concentrations over the range used. The day-to-day drift in the calibration line was very slight.

In preliminary experiments with aqueous aneurine solutions, precipitation of mercuric oxychloride (as mentioned by Holman) sometimes occurred. Although the precipitate dissolved after the addition of acetone, results in these instances were low and erratic. Occasionally mercuric oxide was also precipitated. Both these difficulties were overcome by reducing the concentration of potassium hydroxide from 0·06 N to 0·04 N, and that of the mercuric chloride solution from 1·0 to 0·8 per cent. With these modifications highly reproducible results were obtained, sixty determinations giving a coefficient of variation of 1·0 per cent.

Reproducible results were obtained with dilute hydrochloric acid extracts of tablets. Eleven assays of samples from one batch of B group tablets carried out over a period of 6 weeks gave results as follows:—

Mean weight of aneurine per tablet	0·958 mg.
Range	0·94 to 0·98 mg.
Coefficient of variation	1·6 per cent.

Satisfactory precision was also obtained with the multi-vitamin concentrate, the aqueous alcoholic solution being first diluted with water and extracted with carbon tetrachloride to remove vitamins A and D.

To test the accuracy of the method extracts from a B group tablet granule without aneurine were prepared for assay in the usual way and measured volumes of standard aneurine solution were added before oxidation. Ten assays were carried out with the following results:

Mean recovery	98·8 per cent.
Range	98 to 102 per cent.
Coefficient of variation	1·6 per cent.

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With the chocolate-malt granules very poor results were obtained by simple acid extraction. Separation of the aneurine from interfering substances by adsorption on synthetic zeolite was therefore investigated.

ADSORPTION OF ANEURINE ON SYNTHETIC ZEOLITE—

The most suitable adsorbent obtainable was a siliceous synthetic zeolite, as used for water purification. It was found that preliminary treatment with 3 per cent. acetic acid, as usually described for Decalso, was not sufficient. Iron, the main impurity, interfered with the assay, because it was precipitated when the solution was made alkaline for oxidation. Removal of the iron by heating under reflux with hydrochloric acid was investigated; but, although all iron could be removed with normal or more concentrated acid, the adsorptive capacity of the zeolite was considerably reduced. Eventually it was found best to activate the zeolite in the columns, by washing alternately with boiling 3 per cent. acetic acid and with a 25 per cent. solution of potassium chloride in 0·1 *N* hydrochloric acid until no precipitate appeared when the potassium chloride washings were made alkaline.

On following the method of Hochberg *et al.*[4] with columns 120 mm. long and 6 mm. in internal diameter, recoveries were found to be low. Investigation proved that aneurine was completely removed from solutions passed through the columns, suggesting incomplete elution. On increasing the volume of eluant to 40 ml. and insulating the columns with asbestos string, satisfactory recoveries were obtained.

To test the performance of these columns with extracts from the chocolate-malt granules, a laboratory batch containing exactly 0·1 mg. of aneurine per gram was prepared. Nine samples were heated under reflux with 0·05 *N* sulphuric acid, the vitamin A, other fatty substances and vanillin being extracted with carbon tetrachloride, and from each extract duplicate aliquots were analysed, with the following results:—

Mean weight of aneurine per gram	0·0995 mg.
Range	0·095 to 0·102 mg.
Coefficient of variation	2·0 per cent.

Columns of this type have been in routine use for several months and continue to function satisfactorily.

METHOD FOR SOLUTIONS AND TABLETS

REAGENTS—

(1) Potassium chloride solution, 25 per cent. w/v in water.

(2) Mercuric chloride solution, 0·8 per cent. w/v in water.

(3) Potassium hydroxide solution, 0·04 *N*.

(4) Acetone, commercial, heated with activated carbon under reflux and distilled in an all-glass apparatus.

(5) 1-Methyl-5-aminoacridine hydrochloride solution, 0·20 μg. of the monohydrate per ml. in water. This solution is prepared, approximately weekly, from one containing 100 μg. per ml. in water. The latter solution, stored in the dark, has so far been stable for two years. The dilute solution in the fluorimeter cell should be changed after about 1 minute total exposure.

(6) Aneurine standard solution, 2·0 μg. per ml. in water. This solution is prepared daily from one containing 100 μg. per ml. in 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid. Aneurine is recrystallised from aqueous alcohol acidified with hydrochloric acid, and dried under vacuum at approximately 75° C.

PREPARATION OF SOLUTION—

(*a*) *Solutions*—Pipette a suitable volume of sample and dilute to give a final aneurine concentration of 0·5 to 2·0 μg. per ml. in 0·01 *N* hydrochloric acid.

(*b*) *Tablets*—Weigh a quantity of finely powdered sample containing about 1 mg. of aneurine and shake with 0·01 *N* hydrochloric acid in a 1-litre standard flask. Make to volume with 0·01 *N* hydrochloric acid and allow insoluble matter to settle. If necessary, centrifuge a small volume to obtain a clear solution.

It may sometimes be necessary to weigh a larger sample and use two dilution stages.

DETERMINATION—

Pipette 1 ml. of solution prepared as above, and aliquots of standard solution containing 0·5 to 2·0 μg. of aneurine, into 25-ml. glass-stoppered measuring cylinders or standard flasks.

Make up the volume to 7·5 ml. with 25 per cent. potassium chloride solution. Add 1 ml. of 0·8 per cent. mercuric chloride solution and mix well. Add 4 ml. of 0·04 *N* potassium hydroxide and mix again. (It is important to mix well before and after addition of the alkali.) Stopper the cylinders or flasks and heat in a water-bath at 40° C. for 15 minutes.

Cool and make up the volume to 25 ml. with acetone. Measure the fluorescence of the solutions, using 1-methyl-5-amino-acridine hydrochloride solution as standard.

METHOD FOR PREPARATIONS CONTAINING CHOCOLATE, MALT EXTRACT, VANILLIN, ETC.

ADDITIONAL REAGENTS—

(1) Potassium chloride in hydrochloric acid solution, 25 per cent. w/v of potassium chloride in 0·1 *N* hydrochloric acid.

(2) Sulphuric acid, 0·05 *N*.
(3) Sodium acetate solution, 1·8 *M*.
(4) Potassium hydroxide solution, 0·1 *N*.
(5) Carbon tetrachloride, redistilled.
(6) Synthetic zeolite (supplied by Fletcher Chemical Co. (Aust.) Pty. Ltd., Melbourne).
(7) Acetic acid, 3 per cent. w/v.

Adsorption apparatus as described by Hochberg *et al.*[4], the columns being insulated with three layers of asbestos string.

PREPARATION AND ACTIVATION OF ZEOLITE—

Grind and sieve a sample of zeolite and collect the 44–85 B.S.S. fraction. Soak the adsorption tube in chromic acid solution for several hours and rinse and dry it thoroughly. Place a small wad of glass wool at the bottom of the column. Fill the dry zeolite carefully into the column, tapping gently to ensure even packing, and wash with water until the washings are clear. Pour 50 ml. of boiling 3 per cent. acetic acid through the column, followed by 100 ml. of 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid (approximately 3 drops per second). Repeat this process until there is no precipitate when the KCl + HCl washings are made alkaline. (Approximate volumes required: 250 ml. of the 3 per cent. acetic acid and 500 ml. of the KCl + HCl solution.)

PREPARATION OF EXTRACT—

Weigh a portion of the ground sample containing about 10 μg. of aneurine and heat under reflux with 150 ml. of 0·05 *N* sulphuric acid for 30 minutes. Adjust the solution to pH 4·0 to 4·5 with 1·8 *M* sodium acetate solution. Make up the volume to 200 ml. and heat the solution under reflux for a further 30 minutes with carbon tetrachloride to remove vitamin A concentrate, cocoa fat and vanillin. Then centrifuge at least 50 ml. of the aqueous layer until clear.

ADSORPTION AND ELUTION OF ANEURINE—

Pass 50·0 ml. of the clear solution through the column at room temperature (not more than 3 drops per second). Pass steam through the jacket and fill the column with boiling water. Allow to heat for ½ minute and then draw through rapidly. Repeat this heating process three times. Elute the aneurine immediately with 40 ml. of boiling 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid (not more than 2 drops per second). Dilute the eluate to 50 ml. with water.

Wash the column with 200 ml. of boiling water, followed by 50 ml. of cold water. It is then ready for the next sample.

ASSAY—

Titrate 5·0 ml. of the prepared solution with 0·1 *N* potassium hydroxide, to the methyl red end-point. Note the volume, *v* ml., of alkali required. Pipette another 5·0 ml. of solution into a 25-ml. stoppered cylinder or standard flask. Add $(4{\cdot}9 - v)$ ml. of 25 per cent. potassium chloride solution and 1 ml. of 0·8 per cent. mercuric chloride solution. Mix well. Add $(v + 1{\cdot}6)$ ml. of 0·1 *N* potassium hydroxide and mix again. Complete the assay as described under "Method for Solutions and Tablets."

SUMMARY

The method of Holman,[4] using mercuric oxide as oxidant, has been adapted for the fluorimetric assay of aneurine in pharmaceutical products. With a preparation containing

chocolate, malt extract and vanillin the aneurine is first separated from interfering substances by adsorption on synthetic zeolite. The method described is simpler, quicker and of greater precision and accuracy than those based on the conventional ferricyanide oxidation.

The authors wish to thank the Directors of Nicholas Pty. Ltd., Melbourne, for permission to publish this paper and Mr. D. C. M. Adamson for reading the proof.

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NICHOLAS PTY., LTD.
MELBOURNE, AUSTRALIA

September, 1948

A Micro Method for the Determination of Unsaturation

BY MISS W. M. PHILLIPS AND W. C. WAKE

(*Read at the meeting of the Society on November 3rd*, 1948)

ALTHOUGH most of the techniques used in chemical analysis have been adapted for use with small or very small quantities of material, no such adaptation of the Wijs procedure for determining unsaturation appears to be recorded. The work described in this paper examines the determination of unsaturation on the micro scale both with iodine chloride and with bromine, and with particular reference to *cyclo*hexene as a standard substance; some values obtained for linseed and castor oils are also recorded. The use of a specially designed "iodine flask" was found necessary and it may be noted that this piece of apparatus may also be used with advantage on the macro scale.

EXPERIMENTAL

The essentials of most halometric methods for measuring unsaturation involve a final titration with sodium thiosulphate of iodine liberated from potassium iodide solution. Attention was, therefore, first concentrated on the estimation of iodine chloride dissolved in carbon tetrachloride, by means of aqueous sodium thiosulphate solution. On the normal scale of working, adequate interaction between the two phases in the course of the titration is usually accomplished by re-stoppering the flask after each addition of sodium thiosulphate and shaking vigorously to re-establish equilibrium between the aqueous and non-aqueous phases. This is not possible on the micro scale because of the necessity, when titrating small volumes, of keeping the tip of the burette below the surface of the aqueous layer while adding solution from the burette. The use of a nitrogen stream, as frequently recommended for micro-titrations, is impracticable because of volatilisation of iodine. The method finally adopted was to use glass beads in the flask, the beads being of such size that, although resting on the bottom of the flask, they projected through the interface of the two phases. It was found that the liquid in the flask could be swirled round sufficiently rapidly for the rolling beads to cause turbulence at the interface, and trials showed that they considerably shortened the time required for the titration.

Comparison was made of the standardisation of iodine chloride in carbon tetrachloride on the macro and on the micro scale. The mean of four micro-determinations was 0·1954 *N* with a coefficient of variation* of 1·7 per cent., whilst four macro-determinations gave 0·1967 *N* with a coefficient of variation of only 0·34 per cent. Further experiments showed differences between the results obtained by carrying out the titration immediately and those obtained after the solution had been allowed to stand in the dark for 1 hour. To investigate the cause of this difference, six titrations were made, three without standing and three after allowing to stand for 1 hour, each set of three consisting of a series in which the volume of diluting solvent (carbon tetrachloride) was 0, 25, and 100 ml. respectively. The addition of carbon tetrachloride had no effect on those titrated immediately, but in those left for 1 hour there was an apparent loss of iodine chloride (*i.e.*, a decrease in normality) greatest for that to which the least solvent had been added. The series was repeated except that, before titrating but after standing, the flasks were cooled in ice to reduce the vapour pressure in them. In this way potassium iodide solution around the stopper was allowed to run in without apparent displacement of vapour. As a result, the loss of iodine was considerably reduced, although some still occurred where no additional carbon tetrachloride had been added.

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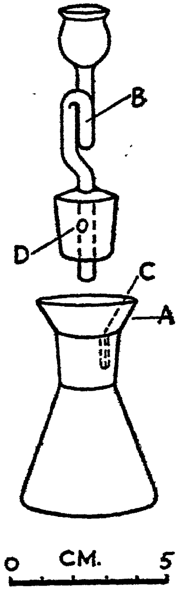
* The coefficient of variation is the standard deviation expressed as a percentage of the mean value of the quantity determined.



These experiments pointed to a loss of iodine chloride as vapour, probably by diffusion from the flask during the operation of adding the potassium iodide solution, the higher loss after standing being due to the extra time, which allowed an equilibrium partial pressure of vapour to establish itself. To overcome this a special micro iodine flask was designed as shown in the Figure. In addition to the usual wide lip, A, for sealing with potassium iodide solution, a centrally placed thistle funnel, B, with a single-turn trap for liquid is added to the stopper. The ground part of the seating for the stopper contains an indentation, C, and the stopper has a hole, D, which can be brought opposite this indentation. When carrying out a determination, after the necessary standing period, the stopper is turned so that the hole and the indentation register, and excess of potassium iodide solution is added through the funnel, any vapour displaced being washed by bubbling through the potassium iodide solution in the lip of the flask. Once there is an excess of potassium iodide in the flask a few minutes suffice for the absorption of any iodine chloride vapour. Experiments showed that no loss occurred on standing and the spread of the titrations was much reduced, as is shown by Table I.

TABLE I

NORMALITY OF IODINE CHLORIDE DETERMINED BY MICRO-TITRATION

Each figure is based on four determinations

Flask used	Normality	Coefficient of variation of normality determination %
Ordinary flask	0·1954 *N*	1·7
Special iodine flask ..	0·00990 *N*	0·9
	0·0159 *N*	0·5
	0·3676 *N*	0·2

The titrations were carried out with 0·1 *N* sodium thiosulphate contained in a horizontal burette about 30 inches long, with a capacity of 1·6 ml. The iodine chloride was, owing to supply difficulties, prepared from its elements in the laboratory.

PROCEDURE ADOPTED

*Cyclo*hexene, which was adopted as a convenient standard, was passed down an alumina column under purified nitrogen to remove peroxide. The chromatographed material was tested for peroxide by the method of Yule and Wilson[1] and glass capillaries were filled in the usual way. The capillaries, containing about 6 mg., were crushed with a glass rod under about 3 ml. of carbon tetrachloride in the iodine flask, which already contained the glass beads needed during the titration. A further 2 ml. of carbon tetrachloride was used to rinse the rod, and the calculated quantity of iodine chloride or bromine solution was added from an automatic burette, a standard piece of apparatus, the graduated portion, 25 ml. in capacity, being fitted on to a 1-litre reservoir by a ground glass joint; solution from the reservoir was pumped up into the burette by means of compressed air. This piece of apparatus was probably the limiting factor in accuracy, as its graduations were 0·1 ml. It is essential to use such a burette for the iodine chloride or bromine solution, not only because of the high vapour pressure of the solution but also because of the need to exclude water vapour.

After the addition of the halogen solution, the stopper, already having some 10 per cent. potassium iodide solution in the liquid trap, was placed in position, and further potassium iodide solution was added to the rim. The sealed flask was then placed in a cupboard away from light for the period required for the reaction. After this prescribed period, the funnel of the flask was filled with potassium iodide solution and the stopper turned so that the solution entered slowly, the displaced air and vapour issuing, a bubble at a time, through the iodide seal in the rim. The flask was then allowed to stand for a minute before the stopper was removed and the titration carried out. After the completion of the titration, potassium iodate solution was added and the titration continued if there was any liberation of iodine. This enabled the occurrence of substitution to be tested and, if necessary, allowed for, since halogen acid would be formed in the substitution reaction in the proportion of 1 equivalent to half an equivalent of the iodine chloride or bromine reagent.[2]

BROMINE VALUES OF *cyclo*HEXENE—

The accuracy of the titration can be assessed from examination of the coefficients of variation for "blank" titrations on the bromine solutions used. These are given in Table II.

TABLE II

Mean normality found	Coefficient of variation %	Number of determinations
0·05011	0·5	3
0·07521	0·2	4
0·03125	1·0	3
0·1181	0·5	4
0·03251	0·5	4
0·03236	0·7	4
0·03228	0·6	3
Mean	0·6	

For a standard reaction period of 1 hour and about 100 per cent. excess of bromine (this ranged from 61 to 112 per cent.), a series of eight determinations was made. No substitution occurred. The values obtained were: 197·0, 194·4, 194·3, 193·0, 194·9, 198·7, 194·1 and 193·3, having a mean of 195·0 compared with the theoretical value 194·6. The coefficient of variation was 1 per cent., showing an experimental error slightly larger than that of the blank determinations. In corresponding determinations on the macro scale two observers found respectively 193·6 and 193·1 for the mean and 0·7 and 1·2 per cent. for the coefficient of variation, based on 7 and 8 determinations respectively.

The effects of variation in time of reaction and excess of reagent were investigated, and it was found that low values are associated only with times as short as 5 minutes and excesses of bromine less than 50 per cent. It would appear that with 100 per cent. excess of reagent the addition reaction occurs so rapidly that it is complete before substitution is perceptible. When bromine is present only in small excess, the reaction slows down as the concentration of the reagent falls, and the velocity of the substitution reaction becomes appreciable compared with that of addition. Even so, such substitution as occurs is equivalent to only 2 or 3 units of the bromine value.

The best conditions are, therefore, a period of, say, 15 minutes with an excess of reagent of the order of 80 to 100 per cent.

IODINE VALUES OF *cyclo*HEXENE—

The iodine values of *cyclo*hexene determined by means of a 100 per cent. excess of a solution of the reagent in glacial acetic acid were found to increase linearly with the length of time of standing. As correction for substitution is not possible in acetic acid medium, further work on this procedure was abandoned and use made only of the reagent in carbon tetrachloride solution.

As with the bromine values, the accuracy of the titration can be assessed from the variation of "blank" values, and this is given in Table III.

The mean coefficient of variation is identical with that obtained for bromine titrations.

Experiments showed that a standard period of 1 hour with 100 per cent. excess of reagent produced substitution, and that 15 minutes with about 60 per cent. excess of reagent was a suitable procedure. Values thus obtained were: 309·6, 309·8, 306·2, 308·4, 309·9 and 308·9, of which the mean value is 308·8 compared with the theoretical figure 309·7. The coefficient of variation was 0·5 per cent. and the agreement of this figure with the corresponding figure for the blanks (see Table III) shows that the precision of the method is largely a function of the measurement and titration of the reagent.

TABLE III

Mean normality found	Coefficient of variation %	Number of determinations
0·02669	0·8	5
0·02619	0·7	4
0·02593	0·4	3
0·02616	0·4	5
0·03170	0·4	4
0·03057	1·1	4
0·1925	0·3	4
0·1937	0·6	4
Mean	0·6	

Attempts were made to use the titrations to investigate the kinetics of the reaction, but the rate of reaction is obviously too great for such simple technique. Table IV shows the values obtained for short reaction times and small excesses of reagent.

TABLE IV

Reaction period, minutes	Excess of reagent %	Iodine value
2	22·5	308·7
2	23·0	308·0
2	20·0	306·9
4	22·5	306·7
4	24·0	308·9
8	23·0	310·7
8	24·7	306·5

IODINE VALUES OF UNSATURATED OILS—

In order to provide evidence of the general applicability of the micro method the iodine values of castor and blown linseed oils were determined under conditions of period of reaction and amount of reagent recommended in a standard work on the subject.[3] With the micro method, carbon tetrachloride was used as solvent, thus permitting correction for substitution, but in some corresponding determinations made by macro procedure the recommendations were strictly adhered to and glacial acetic acid was used as the medium. The results, given in Table V, show reasonable agreement between micro and macro results for castor oil, but for the blown linseed oil this is only true of the total iodine value, for, when corrected for substitution, the micro values are significantly lower than the macro values. The spread of results has not been investigated, nor has the question of removing peroxides.

TABLE V

Material	Reaction period	Excess of reagent %	Iodine value (macro method in acetic acid)	Total iodine value (micro in CCl_4)	Iodine value (micro after correction for substitution)
Castor oil (iodine value expected, *c.* 84)	60 min.	*c.* 100	82·6	88·0	No substitution
			84·5	87·1	
			84·5	85·9	
Means	..	.. ..	83·8	87·0	
Blown linseed oil (iodine value expected, less than 120)	3 hr.	*c.* 100	107·2	105·3	98·2
				106·8	103·5

CONCLUSIONS

The use of special iodine flasks has enabled iodimetric titrations to be carried out with a horizontal micro-burette and about 1 ml. of sodium thiosulphate solution. The application of this to the bromine and iodine values of *cyclo*hexene demonstrates the accurate determination of iodine values with samples of a few milligrams, the accuracy obtained with iodine chloride being that of the volume measurements. Conditions under which full saturation of *cyclo*hexene is achieved without substitution have been found, but these may not be suitable for other olefines. Of more general interest is the application to unsaturated oils, where agreement is obtained with macro procedure if the total iodine value is accepted, but it is shown that with a blown linseed oil some substitution occurs.

The authors express their thanks to Miss S. M. Lanham for carrying out many of the titrations and to the Research Association of British Rubber Manufacturers for permission to publish this paper.

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THE RESEARCH ASSOCIATION OF BRITISH RUBBER MANUFACTURERS
105–107 LANSDOWNE ROAD
CROYDON, SURREY

May, 1948

DISCUSSION

The PRESIDENT congratulated the authors on their paper and enquired whether they had made any comparison with micro-determinations using iodine chloride dissolved in glacial acetic acid.

Mr. C. L. HINTON pointed out that there were already in existence a large number of figures for fatty oils, published and unpublished, that had been obtained by the traditional method; even if these should not be strictly accurate, an undesirable confusion might arise if different figures, obtained by a micro method, should come into use.

Mr. K. A. WILLIAMS asked if a sufficient excess of the reagent was used to leave more unabsorbed at the end of an experiment than was consumed by the fat.

Dr. ROMAN asked whether the chloride ion formed would interfere with the iodate titration.

Mr. WAKE, in reply, thanked the President for his remarks and said that comparative figures with glacial acetic acid in the micro method had not been obtained owing to the uncertainty whether substitution would occur. He agreed that such comparison should have been made with castor and linseed oils. In reply to Mr. Williams, he said that the excess used would leave about an equal quantity. In reply to Dr. Roman, he said that as far as he knew the standardisation of hydrochloric acid by iodate was satisfactory and hence one would not expect interference by the chloride ion.

A Colorimetric Method for the Estimation of Amyl Acetate Vapour in the Air

BY H. M. CUSTANCE AND M. HIGGINS

A CONVENIENT and simple method was required to estimate the amyl acetate content of air in a room used for the extraction of penicillin. Most of the methods previously described entailed absorbing the amyl acetate in ethyl alcohol, hydrolysing and then titrating with standard sulphuric acid the excess of sodium hydroxide used in the hydrolysis.[1,2,3] This type of method is tedious, is not specific and would not in general be capable of giving accurate results in the routine determination of low concentrations of ester vapour. Methods applicable generally to solvent vapours can be used,[1] but are even less specific then the saponification procedure.

Several colour tests for amyl acetate have been described,[1] the best known of which is the Komarowsky reaction; this has been used for the colorimetric determination of amyl acetate and of higher alcohols in fusel oil.[4,5] This method is based on the formation of coloured products through the interaction of higher alcohols and esters with cyclic aldehydes in the presence of concentrated sulphuric acid. The aldehydes which have been employed as reagents include salicylaldehyde, benzaldehyde, *p*-dimethylaminobenzaldehyde, furfural, veratric aldehyde and vanillin. Komarowsky's was considered the most promising method for rapid and convenient determination of amyl acetate in the air. Snell[4] describes the application of the method and includes a comprehensive bibliography. Its application to fusel oil determinations is discussed at some length by Penniman, Smith and Lawshe.[5] Nowhere however are sufficiently detailed figures available for accurately defining the conditions under which the determination of amyl acetate in alcoholic solution may best be made.

We have therefore tested various aldehydes as to their suitability and have developed a method using *p*-dimethylaminobenzaldehyde. The optimum conditions for making determinations by this method have been established and figures compiled for estimating the efficiency of ethyl alcohol as an absorbent for amyl acetate.

The experimental investigations of the conditions for carrying out the method were all made with pure *iso*-amyl acetate prepared from *iso*-amyl alcohol.

DEVELOPMENT OF THE COLOUR—

Selection of suitable solvent—In testing the suitability of a solvent, 2 ml. of a solution containing 0·5 mg. of amyl acetate per ml. of the solvent were treated with 2 ml. of water, 10 ml. of concentrated sulphuric acid and 2 ml. of a 2 per cent. *p*-dimethylaminobenzaldehyde solution in the same solvent. The colour was read in a Spekker absorptiometer after 30 minutes. The choice of solvent was restricted to liquids miscible with water and not decomposed by sulphuric acid. *Cyclo*hexanol and dioxan were unsuitable because they formed a black colour with the reagents. Of the range of solvents tested, ethyl alcohol and methyl alcohol were found the most satisfactory. With alcohols of ordinary laboratory-reagent quality the "blanks" were very high, owing, no doubt, to the presence of higher alcohols. Although a single straight distillation did not improve the solvents in this respect, blanks as low as 0·06 (Spekker reading) with methyl alcohol and 0·33 with ethyl alcohol were obtained by careful fractionation of the solvent. Ethyl alcohol was eventually chosen in preference to methyl alcohol, as its lower volatility made it more suitable for long sampling periods.

Selection of a suitable aldehyde—Two ml. of a solution containing 0·5 mg. of amyl acetate per ml. of alcohol were treated with 2 ml. of water, 10 ml. of concentrated sulphuric acid, and 2 ml. of a 2 per cent. solution of various aldehydes in fractionated ethyl alcohol (see page 310) and the colour was read after 30 minutes. A blank was prepared and read at the same time. With salicylaldehyde, crotonaldehyde and paraldehyde, the blank was too dense to be read with the Spekker absorptiometer. When benzaldehyde was used the solution was cloudy. With vanillin the readings for the blank and standard solutions were respectively 0·50 and 1·45, and with *p*-dimethylaminobenzaldehyde 0·34 and 1·70. The latter substance was selected.

Optimum concentrations—The colour intensity was found to be reduced by water, but with the water content below a certain amount the colour of the blank became too intense. The addition of 2 ml. of water to 2 ml. of sample solution gave the best results. Alcohol also reduced the colour intensity, so that an optimum value existed for the amount of sample solution to be employed; this was found to be 2 ml. A 2 per cent. solution of *p*-dimethylaminobenzaldehyde gave the most satisfactory results.

TABLE I

EFFECT OF TEMPERATURE ON THE COLOUR REACTION

Type of reaction vessel	conical flask, 50 ml.	conical flask, 150 ml.	conical flask, 250 ml.	Test tube, 9 × 1 in.
Maximum temperature	84° C.	74° C.	58° C.	82° C.
Average temperature during 30 minutes	40° C.	34° C.	29° C.	44° C.
Absorptiometer reading after 30 minutes:				
Total reading for 0·3 mg. of amyl acetate	0·69	0·40	0·28	0·71
Blank reading	(0·35)	(0·29)	(0·20)	(0·32)
Net reading	0·34	0·11	0·08	0·39
Absorptiometer reading after holding for 30 minutes in a water-bath at 60° C.:				
Total reading for 0·3 mg. of amyl acetate	1·03	—	—	1·07
Blank reading	(0·59)	—	—	(0·59)
Net reading	0·44			0·48

As the volume of sulphuric acid added was increased, so the colour intensity rose to a maximum at 15 ml., beyond which amount it slowly decreased. The volume selected was 10 ml., as it was considered desirable to keep the volume of the reagents small, and the sensitivity of the method was such that the increase in colour of about 50 per cent. that would occur if 15 ml. were used instead of 10 ml. was not of practical importance.

Effect of temperature—The early determinations were carried out by adding the reagents to the alcoholic solution of amyl acetate in 50-ml. conical flasks, allowing them to cool and then making the absorptiometer readings. Consistent results were difficult to obtain, particularly when flasks of different dimensions were used. This was traced to differences in temperature produced in flasks of different sizes when the sulphuric acid was added to the mixture.

Table I shows these different temperatures, together with two sets of absorptiometer readings, the first taken after the reaction vessels had been allowed to stand for 30 minutes, the second after they had been held for 30 minutes in a water-bath at 60° C. instead.

As high temperatures led to very high blanks it was decided to hold the vessels in a water-bath at 60° C. The time of heating, while not particularly critical, did affect the results. The extent of this effect can be seen from Fig. 2, in which time of heating at 60° C. is plotted against absorptiometer readings, both for a blank and for a solution of 0·5 mg. of amyl acetate per ml. of alcohol. Extending the time of heating beyond 30 minutes not only rendered the method more inconvenient but also increased the blank reading without appreciably increasing the sensitivity of the method. If a quicker procedure were desired, satisfactory results could be obtained after 10 minutes' heating.

Stability of colour—The reaction was carried out as outlined below (page 313), and after the heating at 60° C. for 30 minutes, the solution was cooled to room temperature and readings of the colour were then made at various intervals. The results in Table II show that the colour is stable for at least 2½ hours, and that the readings may be made within 5 minutes of removal from the water-bath.

TABLE II

STABILITY OF COLOUR

Time after removal from bath, min.	Readings for 0·3 mg. of amyl acetate present	Blank readings
5	1·26	0·70
30	1·28	0·72
60	1·29	0·72
90	1·29	0·72
150	1·29	0·72

Details of the method finally adopted for determining amyl acetate in air are set out below. The amyl acetate is absorbed in fractionated ethyl alcohol and determined by the colorimetric procedure worked out as described above.

METHOD

APPARATUS—

The amyl acetate vapour was absorbed by means of two simple all-glass bubblers (6 in. by 1 in.), of the design shown in Fig. 1, connected in series. An electric pump and rotameter were used for drawing air through these bubblers at a rate of 0·5 litre per minute.

A Spekker absorptiometer with 1-cm. cells and equipped with Ilford 603 filters was used for measuring the coloured solutions.

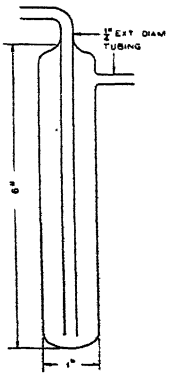

Fig. 1. Absorption Bubbler

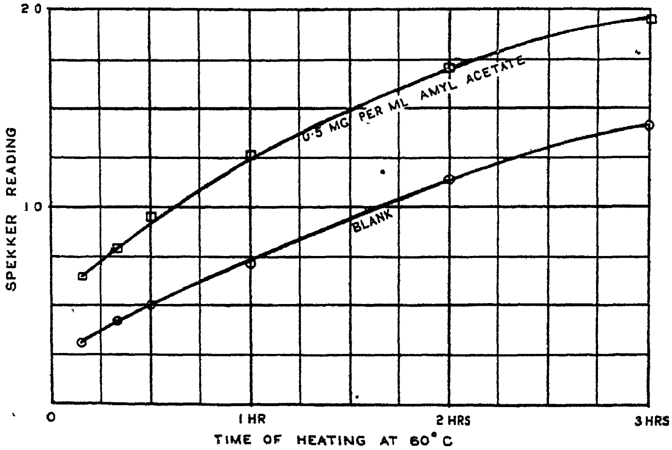

Fig. 2

REAGENTS—

Fractionated absolute alcohol—Absolute ethyl alcohol fractionated through a 3-ft. Stedman column or its equivalent.

Reagent solution—2 g. of *p*-dimethylaminobenzaldehyde in 100 ml. of fractionated absolute alcohol.

Sulphuric acid—C.P. sulphuric acid (about 96 per cent.).

Standard solution of amyl acetate—0·50 g. of amyl acetate made up to 1 litre with fractionated absolute alcohol. The amyl acetate used for making up this standard should be obtained from the same source as that producing the atmospheric contamination.

PROCEDURE—

The air to be tested was drawn through the bubblers, each charged with 10 ml. of the fractionated absolute alcohol, at a rate of 0·5 litre per minute for a sampling period of 30 minutes. The bubblers were then washed out separately with the fractionated absolute alcohol and each solution was made up to a volume of 15 ml.

To 2 ml. of the resulting solution from a bubbler were added 2 ml. of water, 2 ml. of the reagent solution and 10 ml. of the sulphuric acid in a 1-in. by 9-in. test tube. The test tube was kept in a water-bath at 60° C. for 30 minutes and then cooled for 5 minutes and the colour read within 2 hours, with distilled water as the comparison liquid.

Standard curves were prepared each day by carrying out the reaction with 0, 0·1, 0·5, 1·0, 1·5, and 2·0 ml. of the standard solution of amyl acetate, to each of which the required amount of the fractionated absolute alcohol to bring the volume to 2·0 ml. was added.

RESULTS

EFFICIENCY OF ETHYL ALCOHOL AS AN ABSORBENT—

A large number of determinations of amyl acetate in rooms used for extracting penicillin have been made by means of this standardised method. From the separate analytical results from each of the two bubblers it was possible to estimate the suitability of ethyl alcohol as an absorbent for amyl acetate. Typical figures are given in Table III. It appears that in the range of concentrations encountered satisfactory absorption took place in two bubblers, since on the average, 91 per cent. of the total amount collected by the two bubblers was collected in the first.

TABLE III

ABSORPTION OF AMYL ACETATE

Weight in first bubbler mg.	Weight in second bubbler mg.	Concentration calculated from analytical figures mg. per cubic metre of air	Percentage of total amount absorbed in first bubbler
53·1	4·8	3860	92
52·8	4·8	3830	92
50·8	3·9	3640	93
49·7	3·9	3560	93
16·4	1·8	1210	90
15·0	1·2	1078	93
13·8	1·5	1018	90
13·1	1·4	965	90
12·5	1·3	920	90
12·1	1·3	892	90
8·55	0·72	617	92
7·97	0·91	591	90
7·40	1·03	561	88
7·12	0·99	540	88
7·05	0·65	513	92

APPLICATION RANGE—

A standardisation curve from 0 to 0·4 mg. per ml., covering the range of readings on the Spekker absorptiometer from 0·3 (the blank) to 1·4, is a straight line. The blank has a rather high value and has been found to vary between 0·3 and 0·7, depending on the ethyl alcohol used. By careful fractionation it can be kept at the lower figure, and for a given batch of alcohol is closely reproducible. The figures in Table IV give the results of five series of seven determinations made with known solutions on different days, all based on the same calibration curve. It can be seen that the method is very satisfactory for concentrations down to 0·05 mg. per ml. of solution.

TABLE IV

ANALYSIS OF KNOWN SOLUTIONS OF AMYL ACETATE

Concentrations of solution mg./ml.	Averages of seven determinations mg./ml.	Standard deviation
0·01	0·017	± 0·005
0·05	0·051	± 0·003
0·10	0·098	± 0·005
0·20	0·199	± 0·006
0·30	0·297	± 0·005

With the recommended procedure, 0·05 mg. per ml. corresponds to a concentration in the air of 50 mg. per cubic metre, or 10 parts per million.

COMPARISON WITH SAPONIFICATION METHOD—

Thirty-seven pairs of determinations were carried out with air containing varying concentrations of amyl acetate. Of each pair, one determination was made by the colorimetric and the other by the saponification method.[1] A statistical analysis was made of these results and, as indicated in Table V, the "t" test showed that there was no significant difference between the means.

TABLE V

DETERMINATIONS OF AMYL ACETATE IN AIR

Method	Number of determinations	Mean	Standard deviation	t	Value of t for probability of 0·05 against
Colorimetric	37	889·5	72·38	0·674	2·03
Saponification	37	855·3	73·62		

A series of alcoholic solutions of amyl acetate of known concentration was prepared and each solution analysed by both procedures. Twenty determinations were made by each method on each of the solutions, and a summary of the results is given in Table VI.

TABLE VI

COMPARISON WITH SAPONIFICATION METHOD

Concentration of amyl acetate taken, mg. per ml.	Found by saponification method		Found by colorimetric method		Comparison between saponification and colorimetric methods	
	Mean mg. per ml.	Standard deviation	Mean mg. per ml.	Standard deviation	Difference between means	t
2	1·89	0·022	1·92	0·081	0·03	0·81
1	1·01	0·020	1·01	0·031	nil	nil
0·5	0·484	0·021	0·490	0·013	0·006	1·9
0·2	0·190	0·020	0·195	0·007	0·005	2·1
For 38 degrees of freedom and $p = 0·05$						2·0
For 38 degrees of freedom and $p = 0·02$						2·4

It is obvious from these results that the colorimetric procedure was not subject to any systematic error. Whilst at concentrations of 1·0 mg. per ml. and higher, results obtained by saponification showed a lower standard deviation than those obtained by colorimetric analysis, the latter method was superior at lower concentrations.

APPLICATION TO *n*-BUTYL ACETATE—

When the procedure for amyl acetate was applied directly to *n*-butyl acetate it was found that approximately 100 times the amount of *n*-butyl acetate was required to give colours of the same density as with amyl acetate. This means that the minimum concentration of *n*-butyl acetate that could be determined with any accuracy by this method would be of the order of 5000 mg. per cubic metre of air or 1000 parts per million. This renders the method unsuitable for the determination of *n*-butyl acetate. It is also apparent that small amounts of *n*-butyl acetate would not affect the determination of amyl acetate.

DISCUSSION AND CONCLUSIONS

According to Snell,[4] the reaction is given, in general, by higher aliphatic alcohols and their esters, hydro-aromatic alcohols, phenols and straight-chain ethylene compounds. However, the amount of interference depends upon the behaviour of the compound under the conditions of the procedure as developed here. From the information given by Penniman, Smith and Lawshe,[5] it would appear that *n*-butyl, *iso*-propyl and *n*-propyl alcohols are unlikely to give serious interference if present in small quantities, but that *n*-amyl and *iso*-butyl alcohols may very well do so, since they are recorded as giving colours of the same intensity as *iso*-amyl alcohol.

The method is capable of determining accurately concentrations of amyl acetate in the air down to 10 parts per million, and is in this respect superior to the saponification procedure. Furthermore, although not completely specific for amyl acetate, the method compares favourably with any other available procedure from the point of view of specificity. Extensive use has confirmed the value of the method and proved it to be convenient and accurate.

SUMMARY

A colorimetric method, based on the colour given by the reaction of amyl acetate with sulphuric acid and *p*-dimethylaminobenzaldehyde in ethyl alcohol solution, has been applied to the quantitative determination of amyl acetate vapour in the air. The optimum conditions for the application of this method have been established, and comparisons made between it and the saponification procedure. For low concentrations the colorimetric procedure was found preferable. As an absorbent for the amyl acetate, ethyl alcohol proved satisfactory.

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COMMONWEALTH OF AUSTRALIA
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May, 1948

Determination of the Iron Content of Blood Serum

BY S. L. TOMPSETT AND R. A. McALLISTER

NORMAL blood serum contains from 50 to 150 μg. of iron per 100 ml. Larger or smaller values may be encountered in disease. This iron appears to exist as a labile compound with protein, as distinct from the more firmly bound iron of the haemoglobin present in the red blood cells. Owing to the small amount of serum usually available for analysis, it is necessary to be in a position to estimate quantities of iron of the order of 1 to 3 μg. with some degree of accuracy. Methods involving a preliminary ashing are to be avoided because serum usually contains some haemoglobin from haemolysed red blood cells. By the use of appropriate techniques, serum iron can be recovered quantitatively in trichloroacetic acid extracts of serum, haemoglobin iron being unextracted (Barkan[1], Barkan and Walker,[2] Tompsett[3]). Such iron may be determined directly in these extracts. One of us[3] employed the violet colour produced with thiolacetic acid and ammonia in conjunction with visual colorimetry. Barkan and Walker[2] described a method in which the depth of colour produced with *o*-phenanthroline was determined in a visual colorimeter. The depth of colour was determined after the reactants had stood at room temperature for at least 1 hour.

The present writers found that colour development with *o*-phenanthroline was exceedingly irregular at room temperature. At 37° C., however, colour development was found to be more regular, and reproducible results could be obtained after 1 hour.

The following is a description of the method now adopted.

METHOD

All glassware must be washed with dilute hydrochloric acid and then with glass-distilled water and dried.

Collection of blood—

About 10 ml. of blood are collected in a centrifuge tube and centrifuged as soon as possible and the serum is separated. An all-glass syringe fitted with a stainless steel needle should be used for collecting the blood.

Reagents—

(1) Hydrochloric acid, 1·2 per cent.

(2) Trichloroacetic acid, 20 per cent. solution.

(3) Sodium acetate solution, saturated.

(4) 2 *M* Acetate buffer solution, pH 4·5. This is prepared by mixing 90 volumes of 2 *M* sodium acetate and 110 volumes of 2 *M* acetic acid.

(5) Hydrazine sulphate, 1 per cent. solution in 2 *M* acetate buffer of pH 4·5. This reagent must be freshly prepared before use.

(6) *o*-Phenanthroline, 0·1 per cent. solution in water.

All solutions must be prepared with glass-distilled water and filtered.

Procedure—

Place a mixture of 4 ml. of the serum and 2 ml. of 1·2 per cent. hydrochloric acid in an incubator at 37° C. for at least 1 hour. After cooling to room temperature, precipitate proteins by addition of 2 ml. of 20 per cent. trichloroacetic acid solution and centrifuge the mixture. As the supernatant fluid tends to be slightly turbid, pass it through a No. 42 Whatman filter paper of minimal dimensions which has been washed with dilute hydrochloric acid and water and dried. To 4 ml. of the filtrate add, in order, 1 ml. of saturated sodium acetate solution, 1 ml. of buffered 1 per cent. hydrazine sulphate solution, 1 ml. of 0·1 per cent. *o*-phenanthroline solution and 3 ml. of water.

Place the mixture in an incubator at 37° C. for 1 hour and evaluate the depth of colour in a Spekker absorptiometer, using Ilford Spectrum Green Filters No. 604.

When 1-cm. cells were used it was found that drum readings (less blank) ranging from 0 to 0·08 were proportional to quantities of iron from 0 to 5·8 μg. in the 2 ml. of serum used in the general analysis.

Notes—A blank should register less than 1 μg. of iron. No difficulty has been experienced when current chemicals have been employed—no additional purifications being required. Anti-coagulants should be avoided, since they may cause interference with the colour development.

Results—

Recoveries of iron added to serum are shown in Table I.

TABLE I

RECOVERY OF IRON ADDED TO SERUM

Results expressed in micrograms of iron per 100 ml. of serum

	Initial iron content	Iron added	Total iron determined	Added iron recovered	
	μg.	μg.	μg.	μg.	%
1	66	58	115	55	95
2	60	117	185	125	107
3	60	146	215	155	106
4	105	58	170	65	112
5	105	117	230	125	107
6	105	146	260	155	106

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INSTITUTE OF PATHOLOGY
ROYAL INFIRMARY
GLASGOW

February, 1948

Notes

THE QUANTITATIVE SEPARATION OF BERYLLIUM FROM ALUMINIUM

(*Read at the meeting of the Society, on Wednesday, February 3rd,* 1949)

THE generally accepted standard method for determining beryllium in presence of excess of aluminium is the fusion of the mixed oxides with sodium carbonate.[1,2] This is based on conversion of alumina to soluble aluminate whilst the beryllia remains unaffected and insoluble on subsequent leaching.

Paradoxically, the usual method for decomposition of beryl is also a sodium carbonate fusion. Osborn,[3] who has recently investigated the reaction, showed that whereas complete decomposition is possible with a beryl to sodium carbonate ratio of 1 : 2, complete insolubility of the beryllium is not attained in any circumstances. In addition, it is well known that the aluminium is not always completely soluble and a second treatment of the insoluble residue with sodium carbonate is generally recommended. In view of these difficulties, an alternative method of separation seems desirable.

PRECIPITATION OF BERYLLIUM FROM CAUSTIC ALKALINE SOLUTION—

The reaction that led to the discovery of beryllium by Vauquelin in 1798 depends on the relative instability of the beryllate. Whereas an alkaline solution of sodium or potassium aluminate is stable on boiling, a solution of the beryllate becomes decomposed with precipitation of beryllia. In 1921, Britton[4] showed that this reaction could be applied quantitatively, but it has never come into general use and is not mentioned in Schoeller and Powell's treatise. To obtain quantitative precipitation of the beryllium the concentration of free alkali must be very small and Britton's method was to add strong sodium hydroxide solution to an acid solution of the aluminium and beryllium until the precipitated hydroxides just redissolved; the solution was then diluted and boiled. Our experience is that this method yields very erratic results, the difficulty lying in the fact that the hydroxides will not dissolve very easily in a small excess of alkali, and unless great care is taken more than the optimum amount will be added. It was noticed, however, that on neutralising caustic alkali solutions of aluminium and beryllium with hydrochloric acid, the beryllium begins to precipitate in an appreciably more alkaline solution than the aluminium. If a solution is known to contain both metals, therefore, the alkalinity can be adjusted by adding sufficient acid to cause the first precipitation of beryllium hydroxide. Subsequent boiling completes the precipitation of the beryllium leaving the aluminium in solution.

Although this method is suitable for solutions that are known to contain beryllium, it is clearly desirable with unknown solutions to have an indicator that gives a colour change at the correct conditions for precipitation of beryllium. Of a number of indicators examined, indigo carmine, although not ideal, was found to be the most suitable. In strongly alkaline solution this dye gives a yellow colour, and on neutralising with acid the colour changes through green to blue. The green-blue colour change is fairly sharp at pH 11·2, but the change from yellow to green is more gradual. It was found that while beryllium hydroxide precipitates in the greenish-yellow solution, aluminium is not precipitated until the blue colour is reached. With unknown solutions a few drops of an aqueous solution of indigo carmine should be added and the solution titrated with hydrochloric acid until there is a slight precipitate or until the solution assumes a definite green colour, whichever occurs first. If on boiling the green solution, no precipitate appears, then beryllium is absent.

EXPERIMENTAL—

Different amounts of a standard beryllium sulphate solution were mixed with 50 mg. of aluminium, added in the form of potassium alum solution, treated with 5 g. of sodium hydroxide and diluted to 200 ml. A few drops of 1 per cent. indigo carmine solution were added and diluted hydrochloric acid (1 + 1) was run in from a burette until a slight permanent precipitate appeared in the yellowish-green solutions. After boiling for 30 minutes the precipitates were filtered off and washed with 1 per cent. sodium chloride solution to which sodium hydroxide solution had been added dropwise until a green colour was produced with indigo carmine. The precipitates were then redissolved in hydrochloric acid and precipitated with ammonia, using litmus paper as indicator (methyl red, which is commonly recommended, does not ensure complete precipitation). The reprecipitated beryllium hydroxide was filtered and washed with dilute ammonium chloride solution to remove any sodium salts and finally ignited to BeO.

Recovery of beryllium was quantitative, as the following results show.

PRECIPITATION OF BERYLLIUM IN PRESENCE OF 50 MG. OF ALUMINIUM

BeO added, mg.	BeO recovered, mg.
140·5	140·0
70·2	70·0
35·1	34·8
14·0	13·9

In presence of larger amounts of aluminium, results are somewhat high with one precipitation from caustic soda solution and a double precipitation is necessary for complete separation. This is illustrated by the following figures, which were obtained by precipitating beryllia from solutions containing 1·0 g. of Hilger H.H.P. aluminium.

PRECIPITATION OF BERYLLIUM IN PRESENCE OF 1·0 G. OF ALUMINIUM

	BeO recovered, mg.	
BeO added, mg.	1 precipitation	2 precipitations
145·0	147·0	144·0
72·5	76·0	71·6

The results shown in the last column were obtained by dissolving the first precipitate in hydrochloric acid, adding excess of sodium hydroxide and precipitating again in the same way. After filtering, a final reprecipitation with ammonia was carried out to eliminate sodium chloride.

SUMMARY—

When a caustic alkaline solution of beryllium and aluminium is neutralised with dilute acid, the beryllium hydroxide starts to precipitate first, and on subsequent boiling precipitation of beryllia is quantitative whilst aluminium remains in solution. Indigo carmine may be used as indicator to show the correct alkalinity. With a large excess of aluminium double precipitation from the caustic alkaline solution is necessary.

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DISCUSSION

Mr. W. H. BENNETT asked if the method had been tested for the separation of small quantities of beryllium of the order of 1 mg., such as would be likely to be encountered in the analysis of rocks. Would the author expect any difficulty in filtering and washing small amounts of precipitate, owing to colloidal effects, and did phosphates interfere with this separation?

Mr. G. H. OSBORN asked whether the author had tested for aluminium in the beryllium precipitate, and for beryllium in the aluminium precipitate. He congratulated the author on what was apparently a very useful method of separation of the two elements.

Mr. COPPINS, in reply, said that he had not used the method for separation of less than about 5 mg. of beryllium and considered that quantities of the order of 1 mg. would be better determined by a photometric method which did not involve separation from aluminium. Apart from the usual difficulties of dealing with a very small precipitate, he would not expect any trouble from colloidal effects, as the α-hydroxide of beryllium which is precipitated from alkaline solution is quite granular in character. Difficulty from this source was more likely during the reprecipitation with ammonia, but provided adequate time was allowed before filtering it should not be serious.

As he was more interested in alloys than in minerals, he had not had occasion to investigate the effect of phosphates.

Some of the beryllia precipitates had been examined spectrographically and no aluminium was detected.

ROTARY STIRRING DEVICES FOR MICRO-TITRATION

As with the reciprocating stirrers previously described,[1] the device shown in Fig. 1 is operated by a filter pump. Although the torque is not great, the high speed of rotation produces excellent mixing in aqueous or similar media.

The T-piece A, forming the body, is about 60 mm. long and has 15 mm. inside diameter. Both ends are closed by corks, the lower of which carries bearing B. This is a 40-mm. length of glass tubing, the ends of which are slightly constricted in the flame until spindle C will just not enter. The spindle, which is half of a No. 15 steel knitting needle, is then ground into the bearing with extra fine emery paste until an easy running fit is obtained. Stop D, which is of 6-mm. outside diameter glass tubing, is closed and flattened at the lower end and a small hole is blown in the wall as shown.

Rotor E, which is cut from an ordinary tapering cork, is about 12 mm. long and of 13 mm. diameter at the larger end. By means of a razor blade, about twelve teeth are cut lengthwise so that a "rachet-wheel" section as shown at (*a*) is produced. The pointed end of the spindle, C, is then thrust axially through the rotor as shown; reasonable errors either in alignment or in tooth-shape have little effect upon efficiency. Before insertion into the bearing, a small brass washer, F, is slipped on the spindle beneath the rotor, to prevent sticking.

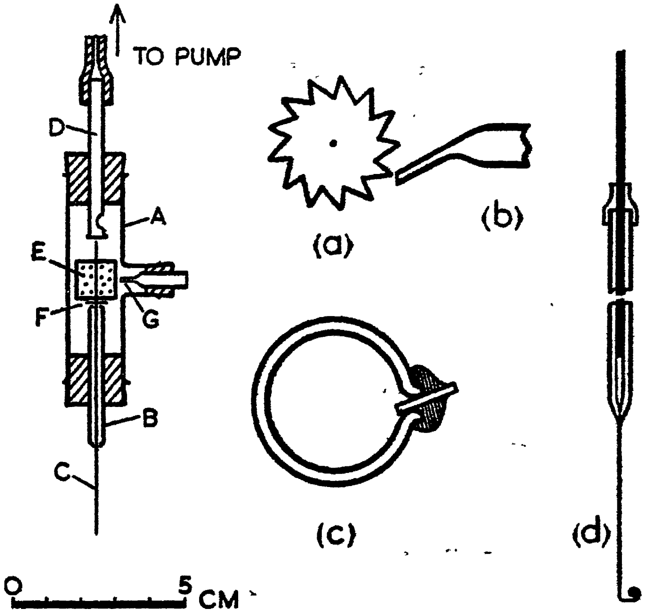

Fig. 1

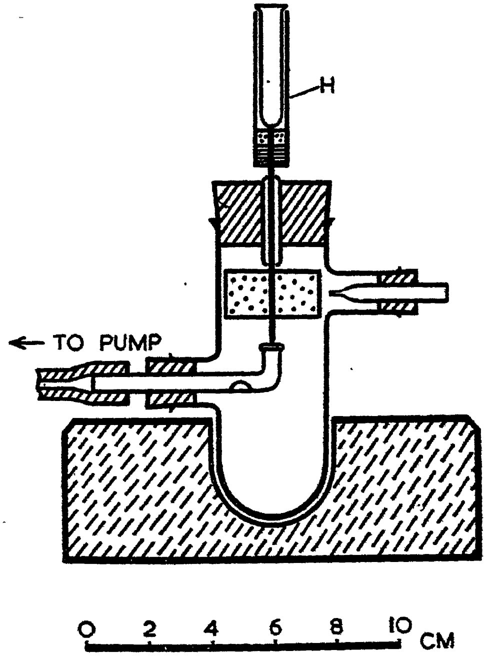

Fig. 2.

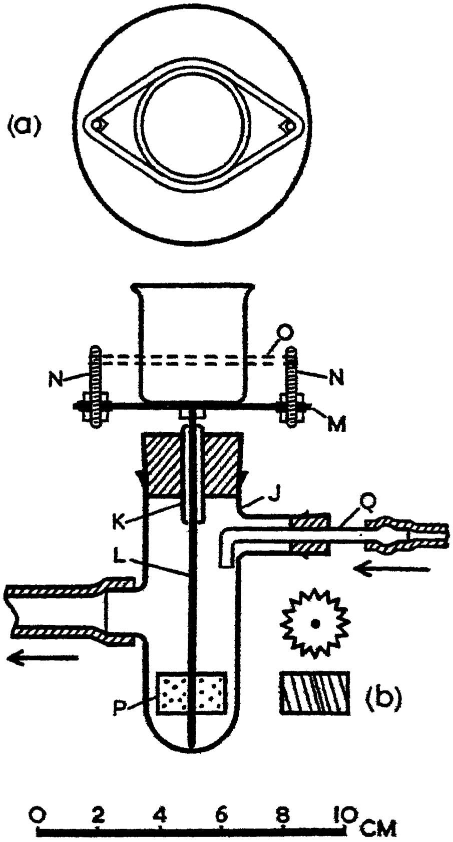

Fig. 3.

Jet G is drawn from 4-mm. outside-diameter tubing to give an orifice of about 1-mm. diameter. It is bent as shown at (*b*), the optimum angle being obtained by trial. An alternative arrangement is to use as a body a piece of glass tubing with a small hole blown midway in the wall; the jet is then cemented in the hole by sealing wax, as shown at (*c*), adjustments being made while the wax is still soft.

The parts are assembled so that the distance between the jet-tip and rotor is 1 to 2 mm., clearance between the pointed upper end of the spindle and the stop being about the same. On connecting to a filter pump, the stream of air drawn in through the jet causes the spindle to rotate rapidly. At the same time, atmospheric pressure slightly lifts the spindle assembly, thus greatly reducing friction.

Interchangeable stirrer-heads as shown at (*d*) are made from wide melting-point tubes, into which the lower end of the spindle slips comfortably. Retention is by a sleeve of cycle valve tubing.

When titrating in narrow vessels, or when, as in potentiometric micro-titration, several pieces of apparatus dip into the solution, a convenient method of stirring is by rotating the vessel.[2,3] For thus performing titrations in a micro test tube, the device shown in Fig. 2 is satisfactory. Construction is along the same lines as with the first device. The rotor, which has about twenty-four teeth, is 30 mm. in diameter. Holder H, a 40-mm. length of glass tubing, is mounted truly on the upper end of the spindle by means of a cork reinforced by sealing wax. The bore of the holder should snugly accommodate the titration vessel, the contents of which may be kept under observation during titration.

For larger quantities of liquid (*i.e.*, from 2 to 25 ml.) a micro-beaker of appropriate size is a more convenient titration vessel. In this case the inherently high speed of these turbine devices is disadvantageous, centrifugal action causing loss of liquid. Some improvement may be achieved by driving by water power instead of air; at low speeds, however, torque is very poor and highly sensitive to mains fluctuations.

The submerged rotor device shown in Fig. 3 was designed to permit steady rotation of micro-beakers at 100 to 150 r.p.m. Container J, of heavy glass, has 27 mm. inside diameter. A rubber stopper carries bearing K, into which spindle L is ground to an easy running fit as described above. The spindle is of 3/32-inch diameter brass rod and is tapered to a rounded point at the lower end. Brass platform M, which is 75 mm. in diameter and about 1 mm. thick, is soldered to the upper end of the spindle, the joint being strengthened by a small brass boss. Two pillars, NN, of No. 4 B.A. brass screw rod, are attached to the platform as shown. A rubber band, O, conveniently made by joining the ends of a length of cycle valve tubing with a short piece of glass rod, is stretched between the pillars. Micro-beakers of different size are held on the platform merely by being slipped within the band, as shown at (*a*).

The cork or rubber rotor P is 23 mm. in diameter and 12 mm. long. It has sixteen teeth, of section as shown at (*b*), cut at an angle of about 15 degrees to the length, and is positioned on the spindle as shown. Jet Q is of 3-mm. bore glass tubing, the end being bent at right angles as shown, but not constricted. The stream of water should be projected vertically downwards as close as possible to the container wall.

In operation the container becomes filled with water so that the rotor is submerged. Liquid drag, which increases rapidly with speed, then hinders rapid revolution. It is peculiar that the rotor turns the opposite way to that expected. The jet of water appears to pass downwards through the annulus formed by container and rotor, so that it is the rising water which causes the rotor to turn. Nevertheless, the direction of rotation may be reversed by turning the stem of the jet through about 45 degrees, thus giving the water a spiral, turbulent path. Under such conditions, however, rotation is more rapid and less uniform. Raising the rotor on the spindle also reduces uniformity of rotation.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

The Official Assay of Ammoniated Mercury Ointment. M. A. Ellis (*Pharm. J.*, 1948, [iv], **107**, 400)—Because of indefinite end-points and consequent variable, low results, a source of error was sought in the B.P. directions for the assay of ammoniated mercury ointment. Difficulty in dissolving all the ammoniated mercury in the prescribed volume of 10 per cent. potassium iodide solution suggested that this quantity is insufficient. For the assay of ammoniated mercury itself it is directed that 0·25 g. of the powder be dissolved in water containing 3 g. of potassium iodide; the ointment contains 2·5 per cent. of medicament so that by analogy, for a 5-g. sample, 1·5 g. of potassium iodide should be required, whereas only 2 ml. of solution (0·2 g.) is stated to be required.

When assays were performed on different samples of ointment, both by strict adherence to the official directions and by addition of 1·5 g. of potassium iodide, the results obtained by the latter method were concordant and reproducible.

The amount of potassium iodide should therefore be increased, probably to 1·5 g. A. H. A. Abbott

Purification of Paraldehyde. C. H. Hunt and F. H. Reuter (*Aust. Chem. Inst. J. and Proc.*, 1948, **15**, 122–125)—Paraldehyde stored in incompletely filled or improperly closed containers is very susceptible to the formation of peroxide impurities, which, in turn, decompose to aldehydes and acids. For 5-ml. samples of paraldehyde the B.P. 1932 gives limits of acid and peroxide impurities as 1·5 ml. of 0·1 *N* sodium hydroxide and 2·0 ml. of 0·1 *N* sodium thiosulphate, respectively, under the conditions of the tests. Samples that do not comply with these requirements can be purified by the destruction of peroxides by heat, extraction with strong caustic soda solution to remove organic acids, and fractionation.

The following procedure, applied to a sample that required 9·7 ml. of 0·1 *N* sodium hydroxide and 1·9 ml. of 0·1 *N* sodium thiosulphate for the acid and peroxide limit tests, resulted in a yield corresponding to 85·5 per cent. of paraldehyde that conformed to the limits laid down by the B.P., in the original material.

Nine litres of the paraldehyde were boiled under a reflux condenser for 2 hr., cooled quickly, and extracted with 500 ml. of a mixture of 1000 ml. of brine and 300 ml. of 10 *N* sodium hydroxide. After washing with two successive 500-ml. portions of brine and allowing the neutral product to stand overnight in a filled closed container it was distilled, using a short fractionating column, and collected in the following fractions:—(1) b.p. 84° to 121° C., (2) b.p. 121° to 123° C., (3) b.p. 123° to 124·5° C., and (4) b.p. 125° C. Fractions (2) to (4) conformed with the B.P. requirements and were mixed and preserved in filled bottles with well-fitting glass stoppers.

Samples stored as described for 6 years showed no appreciable deterioration on re-test.

Samples with initially different acid and peroxide contents may require final collection in different fractions and each fraction should be tested for conformity to the B.P. tests before blending.

A. H. A. Abbott

Biochemical

Colour Test for Tryptophan. H. Tauber (*J. Amer. Chem. Soc.*, 1948, **70**, 2615)—*Procedure*—Suspend 0·5 mg. of tryptophan or 10 mg. of a tryptophan-containing protein in 1 ml. of water, add 3 ml. of perchloric acid (70 to 72 per cent.), and mix well. If a stable, intense yellowish-green colour and fluorescence develop within a few minutes, tryptophan is present. To confirm, add 0·1 ml. of a 1 per cent. aqueous solution of ferric chloride, and note that the yellowish-green colour changes to reddish-orange.

The following tryptophan-containing materials give the reaction: casein, albumen, human blood serum, pepsin, and crystalline soya bean trypsin inhibitor.

The following amino acids do not give the reaction: glycine, alanine, leucine, *iso*leucine, valine, phenylalanine, tyrosine, cysteine, cystine, methionine, threonine, proline, hydroxyproline, histidine, arginine, lysine, serine, aspartic acid, glutamic acid, and *p*-aminobenzoic acid. Indolyl acetic acid, however, gives a slight pink colour and a slight fluorescence. A. H. A. Abbott

Estimation of Leucine in Complex Mixtures of Amino Acids by Reaction with Sodium Hypochlorite. J. Asselineau (*Bull. Soc. Chim.*, 1947, 1065–1068 M)—Earlier work (Aubel and Asselineau, *Ibid.*, 1947, **41**, 689) on the determination of leucine, alanine, and valine by reaction with sodium hypochlorite to form aldehydes is summarised, and the determination of leucine in mixtures of amino acids is described.

Leucine in presence of alanine and valine—Treat the solution, containing about 30 mg. of amino acids, at 0° C. with an excess of *N* to 0·2 *N* sodium hypochlorite. After 10 min. add freshly-prepared 10 per cent. urea solution to remove the excess of hypochlorite. Stir and cool in ice for 10 min. Add the solution of chloramine derivatives dropwise to 60 ml. of boiling 0·1 *M* phosphate buffer (pH 8·5), and remove the aldehydes by a stream of air into bisulphite solution. An apparatus similar to that used for the determination of lactic acid is recommended (*cf.* Lieb and Zacherl, *Z. Physiol. Chem.*, 1932, **211**, 211). Determine the mixed aldehydes (valeric aldehydes, acetaldehyde, and *iso*butyraldehyde, respectively) by titration with 0·01 *N* iodine in the usual way.

The acetaldehyde, representing the alanine, is determined colorimetrically by means of sodium nitroprusside and piperazine.

The *iso*butyraldehyde, representing the valine, is determined (*loc. cit.*) on a new portion of the solution by sweeping the aldehydes into carbon tetrachloride absorbers and heating the carbon tetrachloride solution under a fractioning column with bisulphite absorbers at the top.

The valeric aldehydes, representing the leucine, are calculated by difference.

Application to a mixture of amino acids—Phenylalanine and methionine give aldehydes, but the formation is not quantitative. These amino acids are removed by adsorption on a column of freshly-precipitated silver sulphide. With mixtures of amino acids an alumina column is used before the silver sulphide to remove amino acids which, although not interfering with the estimation, tend to saturate the silver sulphide and prevent complete adsorption of the phenylalanine.

Glycine gives rise to formaldehyde, which is removed by addition of 10 ml. of 1 per cent. asparagine solution to the phosphate buffer.

The method provides for rapid estimation of leucine in mixture of amino acids with an accuracy of about 90 per cent. Alcohol and other substances oxidisable to aldehydes must be absent. Leucine and *iso*leucine are both determined as leucine and cannot be distinguished. V. M. Bond

Determination of Mannitol in Plasma and Urine. A. C. Corcoran and I. H. Page (*J. Biol. Chem.*, 1947, **170**, 165–171)—The principle of the method is the oxidation of mannitol to formic acid and formaldehyde by periodic acid. The conditions of the oxidation are such that glucose produces little formaldehyde. The periodic acid is reduced to iodine by stannous chloride and the formaldehyde produced is determined by the method of MacFadyen (*Ibid.*, 1945, **158**, 107).

Reagents—Periodic acid reagent—0·03 M potassium periodate in 0·25 M sulphuric acid. *Stannous chloride*—Prepare freshly each day and make approximately 0·125 M in 0·3 N hydrochloric acid. Immediately before use take 10 ml. of stannous chloride, add 5 ml. of concentrated hydrochloric acid and titrate against the periodic acid reagent, using starch as indicator. Dilute the stannous chloride so that 10 ml. ≡ 10·2 ml. of the periodic acid reagent. *Chromotropic acid*—Dissolve 0·2 g. of chromotropic acid (1 : 8 dihydroxynaphthalene-3 : 6-disulphonic acid) in 4 ml. of water in a 100-ml. flask and dilute to volume with 15 M sulphuric acid.

Procedure—For plasma samples prepare a 1 : 20 dilution by the method of Somogyi (*Ibid.*, 1945, **160**, 69). Filtrates made with zinc sulphate and sodium hydroxide or cadmium sulphate and sodium hydroxide are equally satisfactory. From a filtrate of mannitol-free plasma prepare two blanks; treat one (oxidised blank) as a sample and treat the other (unoxidised blank) with stannous chloride before adding the periodic acid.

Dilute urine samples with water.

Into a tall test tube graduated at 25 ml., pipette 2 ml. of plasma filtrate or diluted urine containing 10 to 60 µg. of mannitol and not more than 140 µg. of glucose. Use 2 ml. of water in another tube to give a reagent blank. Add 0·5 ml. of periodic acid reagent and allow to stand at room temperature for 8 to 10 min. Add 0·5 ml. of the stannous chloride solution and shake well. Stannic acid is precipitated, and iodine may be formed momentarily. Add 5 ml. of the chromotropic acid reagent from an automatic pipette with free delivery, shaking well. The stannic acid should have dissolved on addition of this reagent. Place the tube in boiling water for 30 min. Remove the tube, cool, make up to 25 ml. and allow to stand at 25° C. in a water-bath. The colour is stable for several hours at this temperature, and the colour intensity changes by about 1 per cent. per ° C. Read the optical density (D) at 570 mµ. in a No. 6–300 cell of a Coleman 6A clinical spectrophotometer, setting the galvanometer at zero with air in the cuvette.

For urine and water samples, find ΔD as:—

$\Delta D = D$ (sample) $- \ D$ (reagent blank).

For plasma samples calculate:—

$\Delta D_1 = D$ (sample) $- \ D$ (unoxidised blank)

$\Delta D_2 = D$ (oxidised blank) $- \ D$ (unoxidised blank).

Read off from a calibration curve the mannitol concentrations corresponding to the ΔD's, and for plasma samples take the mannitol concentration as the difference in the mannitol concentrations corresponding to ΔD_1 and ΔD_2 respectively.

With careful control of experimental technique, the recovery of mannitol with water and urine dilutions is complete with an error of less than 2 per cent. The recovery from plasma filtrates is 98 to 100 per cent. when the glucose content is within the specified limits.

Fructose, α-glycerophosphate, ethylene and propylene glycols, and most compounds containing a –CHOH–CH_2OH or a –CO–CH_2OH grouping would interfere by producing formaldehyde, if they were present in large enough concentrations. Substances with a –CHOH–CHOH– or a –CO–CHOH– or a –CO–CO– group might interfere by using up periodic acid reagent, but would not produce formaldehyde. The interference from glucose is small because in acid solution glucose exists mainly as the pyranose form, which does not have a free –CHOH– group adjacent to the terminal –CH_2OH group. W. S. Wise

Determination of Micro-Quantities of Citric Acid in Biological Fluids. S. Natelson, J. K. Lugovoy, and J. B. Pincus (*J. Biol. Chem.*, 1947, **170**, 597–605)—Hitherto there has been no method of determining citric acid sufficiently sensitive to be applied to less than 20 ml. of blood. The most promising method depends on bromination of citric acid to yield pentabromoacetone which, after extraction with light petroleum, forms a colour when added to sodium sulphide solution. The optimal conditions for this estimation have been determined; 2 to 60 µg. of citric acid in 0·3 ml. of whole blood can be estimated.

The absorption curve of the colour shows a maximum at 450 mµ. This value is different from the values adopted by various other investigators who did not report the absorption curve.

The colour fades with time but the fading is greatly reduced by lowering the temperature to about 0° C. The colour then takes about 15 min. to develop. The addition of pyridine to stabilise the colour is unnecessary.

At 650 mμ., where the absorption should be zero, there is an opalescence due apparently to emulsification of the light petroleum with the aqueous layer. This effect could be eliminated by centrifuging.

The separating funnels of the original method can conveniently be replaced by glass-stoppered test tubes. The phases were separated by centrifuging, and aliquots taken for the next stage. This procedure ensures good separation of the phases so that only one extraction is necessary. This technique could be applied to other methods where separating funnels are used, and washing is necessary.

Reagents—Use A.R. reagents throughout. (1) Standard solution of 1 mg. of anhydrous citric acid per ml. (2) Six per cent. hydrogen peroxide solution—Dilute 10 ml. of 30 per cent. hydrogen peroxide to 50 ml. with water. Keep in a refrigerator. (3) Four per cent. sodium sulphide solution. Dissolve 40 g. of sodium sulphide crystals ($Na_2S.9H_2O$) in 1 litre of water. Discard this reagent if the solution shows a transmission less than 95 per cent. of that of water at 450 mμ. Store the solution in a refrigerator. (4) Light petroleum, b.p. 90° to 100° C. The commercial product must be purified before use. Shake about 700 ml. with 100 ml. of concentrated sulphuric acid in a separating funnel and set aside overnight. Remove the petroleum layer and wash it three times with 50-ml. portions of sulphuric acid, then several times with 100 ml. of water, and finally with a saturated solution of potassium permanganate in 0·5 *N* sulphuric acid. Allow to stand for about 30 min. Wash again with water to remove permanganate. Test a small portion with concentrated sulphuric acid. If a colour develops repeat the treatment with sulphuric acid and permanganate. Dry the purified petroleum over anhydrous potassium carbonate and filter through glass-wool into a distilling flask. Distil and collect the 90° to 100° C. fraction.

Commercial *n*-heptane, b.p. 96° to 97° C., can be used without purification.

Procedure—Pipette 1 ml. of blood, plasma, or serum, or 0·02 ml. of urine into a 15-ml. centrifuge tube. Add rapidly 5 ml. of 10 per cent. trichloroacetic acid by blowing down the pipette, allowing to drain at the end. This is to precipitate the protein as fine particles. Shake and then set aside for 10 min. Centrifuge and place a 5-ml. aliquot of the supernatant fluid in a 15-ml. glass-stoppered graduated centrifuge tube. Add 0·2 ml. of 18 *N* sulphuric acid and shake the tube. Put two hollow glass beads in the tube and place it in an oil-bath at 110° to 120° C. until the volume is reduced to about 2 ml. Cool to room temperature and add 0·2 ml. of saturated bromine water. Allow to stand for a few minutes. The colour due to the excess of bromine should be present. Add 0·2 ml. of 1 *M* potassium bromide, make up to 3 ml. with water, and then add 1 ml. of 1 *N* potassium permanganate. Allow to stand at room temperature for 10 min. and then cool to 10° C. Keeping the tube cold, remove the colour with the minimum amount of 6 per cent. hydrogen peroxide. Dilute to 5 ml. with water. Add exactly 5 ml. of the light petroleum, stopper the tube and shake for 5 min. in a machine. Then centrifuge for 5 min. at 2000 r.p.m. Take a 4-ml. aliquot without disturbing the aqueous phase. Place the aliquot in a 10- or 15-ml. glass-stoppered centrifuge tube and cool in a refrigerator. This solution can be kept overnight in the refrigerator. After this point keep all solutions below 15° C. Add exactly 3·5 ml. of 4 per cent. sodium sulphide solution. Stopper the tube, shake for 1 to 2 min. and then centrifuge for 3 min. at 2000 r.p.m. Pack the centrifuge tube in ice to keep the temperature low. Aspirate off the light petroleum and take about 3 ml. of the coloured aqueous phase, which should fill the cuvette. Read the transmission in a Coleman spectrophotometer at 450 and 650 mμ. from 15 to 35 min. after the sodium sulphide solution was added. Read the colour against the sodium sulphide solution. Subtract the optical density at 650 mμ. from the optical density at 450 mμ.

Prepare a standard curve with dilutions containing 10 to 60 μg. of citric acid per ml. Use 1 ml. of each solution. Prepare a new curve for each new batch of sodium sulphide solution. Use the same procedure as for blood, starting with the addition of sulphuric acid. Omit the preliminary boiling and treatment with bromine water.

The standard curve shows a slight deviation from Beer's law; 2 to 60 μg. of citric acid can be determined with an accuracy to within 5 per cent.

With blood samples, nearly all the citric acid is in the plasma or serum and the clotting mechanism does not remove a measurable amount of citric acid from the serum. Serum should be used in routine determinations. W. S. Wise

Effect of Temperature on the Fading of the Carr - Price Colours of Vitamin A and Common Carotenoid Pigments. M. J. Caldwell and J. S. Hughes (*J. Biol. Chem.*, 1947, **170**, 97–103)—The well known transient blue colour produced by a chloroform solution of antimony trichloride (Carr - Price reagent) with vitamin A and various carotenoid pigments has been investigated.

Vitamin A was used as the alcohol, the crystalline acetate, and the liquid concentrate of the natural esters. There were no major differences in the initial intensity or rate of fading with the various forms used. The maximum colour was reached almost instantaneously and was independent of the temperature. The rate of fading was greatly increased by a rise in temperature. The usual variations in room temperature would probably not greatly affect the Carr - Price determination of vitamin A when the colour is measured within a few seconds of mixing the reactants.

The pigments investigated were α-, β-, and γ-carotene, lycopene, cryptoxanthin, lutein, and zeaxanthin. With these, there was a slower colour development and also a colour fading with time. The rapidly increasing portion of several of these curves coincided with the time of colour measurement in the Carr - Price determination of vitamin A. A high concentration of carotenoid pigments would thus interfere with the estimation. The rate of colour formation from the carotenoid pigments and the Carr - Price reagent is decreased by lowering the temperature and this might be used in a method of estimating vitamin A in presence of a large concentration of carotenoid pigments.

W. S. Wise

Critical Study of the Proposed Modifications of the Roe and Kuether Method for the Determination of Ascorbic Acid, with further contributions to the Chemistry of this Procedure. M. B. Mills and J. H. Roe (*J. Biol. Chem.*, 1947, **170**, 159–164)—Bolomey and Kemmerer (*Ibid.*, 1946, **165**, 377) proposed using glacial acetic acid instead of 85 per cent. sulphuric acid as a reagent to develop colour in the Roe and Kuether method of estimating ascorbic acid (*Ibid.*, 1943, **147**, 399). The use of acetic acid, however, may lead to serious error, owing to the fact that substances other than dehydro-ascorbic acid, or closely related compounds, can couple with the 2 : 4-dinitrophenylhydrazine reagent and produce an interfering colour. With sulphuric acid, these interfering colours fade and after 30 min. are negligible. In acetic acid solution, however, this fading does not occur.

Bolomey and Kemmerer failed to control the redox potential with, *e.g.*, thiourea as proposed in the original method. This accounts for some anomalies observed by them.

The 540-mμ. filter is better for general work, but absorption is greater at 520 mμ. and this wavelength could be used when little or no interfering substance is present, *e.g.*, in determining ascorbic acid in blood.

W. S. Wise

Estimation of Potassium in Composts and Sewage Sludges. P. Garrick (*Nature*, 1947, **160**, 434)—Several methods for the extraction of potash in organic substances containing large quantities of siliceous matter have been tested. The only method found to be satisfactory was to ignite the material and sinter the ash by the Lawrence - Smith method. The potash was then separated by one of the standard methods. In the estimation of potash in sewage sludges and composts very low results may be obtained if a procedure other than that indicated above is followed.

B. Atkinson

Determination of Potassium in Soil and Plant Material by the Cobaltinitrite Method. J. ten Have (*Chem. Weekblad*, 1948, **44**, 484–488)—In this determination the precipitate contains both sodium and potassium in proportions that vary with the conditions of precipitation, although all the potassium is present in the precipitate. In order to know the correct factor, the conditions of precipitation, in particular the temperature, must be carefully observed. The method recommended is as follows.

Procedure for soil extracts—Dry down a portion of the extract with 4 ml. of 20 per cent. ammonium nitrate solution, and ignite to remove organic matter. Dissolve the residue in hydrochloric acid, evaporate the solution to dryness again, and redissolve with addition of a few drops of hydrochloric acid. Treat the solution with ammoniacal ammonium carbonate solution to remove alkaline earths, etc., evaporate the filtrate to dryness, and remove ammonium salts by heating the residue. Dissolve in warm water, filter through a small filter, and evaporate to dryness in a 50-ml. beaker. To the residue add 10 ml. of water and 5 ml. of saturated sodium chloride solution, place the solution in a thermostat at 25° C., and, after 30 min., add 5 ml. of cobaltinitrite reagent. Keep in the thermostat overnight, and then filter through a sintered-glass crucible (porosity G4), washing with the minimum quantity of 10 per cent. acetic acid, and then once with water. The total volume of washing liquor should be 10 to 12 ml. Wash twice with small quantities of 96 per cent. alcohol and twice with acetone, and dry for 30 min. at 105° C.; 100 mg. of precipitate are equivalent to 17·95 mg. of K_2O. If the result is to be obtained by titration, then transfer the crucible containing the precipitate (not washed with alcohol or acetone) to a 250-ml. beaker containing 50 ml. of water, add 5 ml. of 10 per cent. sulphuric acid, followed immediately by an excess of potassium permanganate solution. Heat to boiling and allow to stand for 10 min. covered with a clock-glass, then add an amount of oxalic acid solution equivalent to the permanganate added. Heat to boiling again, and titrate the excess of oxalate with permanganate. The factor varies somewhat with the quantity of potassium; thus 1 ml. of 0·1 *N* potassium permanganate solution corresponds to 0·770 mg. of K_2O if the total quantity of the latter is 15·79 mg.; and to 0·747 mg. for a total of 3·16 mg.

Procedure for plant material—Ash 5 g. of material in a platinum dish at 300° C. Dissolve the ash in hydrochloric acid, transfer to a 100-ml. measuring flask, and heat the solution to boiling. Add milk of lime until the reaction is alkaline, and place in boiling water for 1 hr. After cooling, dilute to 100 ml. with water and filter. Take 20 ml. of the filtrate in a 50-ml. beaker, acidify with acetic acid, and evaporate to dryness. Dissolve the residue in 10 ml. of water and treat as in the previous method.

Preparation of the reagent—*Solution A*—Dissolve 50 g. of hexahydrated cobalt nitrate in 100 ml. of water and 25 ml. of glacial acetic acid. *Solution B*—Dissolve 50 g. of sodium nitrite (potassium-free) in 100 ml. of water. Prepare the reagent by mixing 3 parts of A and 5 parts of B, drawing air through the mixture for at least 90 min., and, on the following day, filtering.

G. Middleton

Water Analysis

Reduction of Potassium Permanganate in the Presence of Glass. C. B. Taylor (*Nature*, 1947, **160**, 56–57)—While performing experiments involving the standard test for decomposable organic matter (*Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents*, H.M. Stationery Office, 1929), it was found that larger amounts of acidified potassium permanganate were reduced in presence of glass beads than in their absence. For investigating this reduction, *N*/80 potassium permanganate containing 25 per cent. by volume of sulphuric acid was used and the procedure of the standard test was followed. With 100-g. quantities of plate, bottle, and Pyrex glass, and silica, crushed to approximately the same size range and dried under identical conditions, amounts of oxygen of the order of 0·7 p.p.m. were absorbed. The amount absorbed was proportional to the weight of glass added. Glass that had been used in the test and then washed with distilled water, but not dried, was not active, but drying at 140° C. or at 30° C. re-activated the glass. Water that had been in contact with active glass for not less than 20 min. reduced potassium permanganate in absence of the glass. B. Atkinson

Organic

Quantitative Determination of Halides in Organic Compounds by the Action of an Alkali Metal in the Medium of an Indifferent Solvent. A. K. Ruzhentseva and V. S. Letina (*J. Anal. Chem. Russ.*, 1948, **3**, 139–146)—For the determination of halogens in organic compounds, neither the original Stepanov method (*J. Russ. Phys.-Chem. Soc.*, 1905, **37**, 12) nor any of the numerous later modifications, *e.g.*, those based on changes in the amounts of alkali metal and alcohol (Bacon, *J. Amer. Chem. Soc.*, 1909, **31**, 49, etc.) and those based on the use of another solvent (Landis and Wichmann, *Ind. Eng. Chem.*, *Anal. Ed.*, 1930, **2**, 394; Umhoefer, *Ibid.*, 1943, **15**, 383, etc.) can be considered as generally applicable. In most cases the method of Landis and Wichmann gives theoretical results, but discrepancies have been found with dichloroethane (12 per cent. low), butyl chloride (15 per cent. low), *p*-chlorophenol (20 per cent. low), and other compounds. The indifferent solvent introduced by these authors was supposed by them to assist in the removal of hydrogen bubbles from the surface of the metallic sodium and thus to maintain its reducing activity. Nevertheless the main reaction is probably not a reduction but an elimination reaction. Hence the possibility of applying the Wurtz - Fittig reaction was studied.

With dibromoethane, 0·1 to 0·3 g., in 50 ml. of xylene, boiled under a reflux condenser for 2 to 3 hr. with 2 to 3 g. of metallic sodium, theoretical results were obtained for the bromine content after addition of alcohol, and water, to the reaction products, removal of xylene, and titration by Volhard's method; but with 1-chloro-2-bromoethane, and dichloroethane, the results were low, as occurred also when a solvent of higher boiling-point was used. Further experiments showed that the sodium (or potassium) must be present in a very finely divided form. Results then obtained for 26 aliphatic, 23 aromatic, and 2 alicyclic compounds were close to the theoretical. In four cases only was a slight modification (see below) necessary.

Procedure—*Preparation of finely-divided sodium*—Place 30 ml. of anhydrous xylene and 2 to 3 g. of sodium, in small pieces, in a pear-shaped flask, insert a cork stopper carrying a glass tube 50 to 70 cm. long, and heat the flask until the xylene begins to boil. Then wrap the flask in a cloth and shake vigorously, using a circular motion until the sodium is reduced to powder.

General procedure—In a 200-ml. pear-shaped flask with a ground joint for a reflux condenser place 2 to 3 g. of sodium or potassium in powder form and 50 ml. of anhydrous xylene. Weigh into a weighing bottle 0·1 to 0·3 g. of the organic substance and introduce the bottle into the flask, rapidly removing the stopper from the bottle and inserting the reflux condenser, the joint being greased with lanoline. No particles of sodium should appear in the neck of the flask. Boil on an electric hot-plate for 2 to 3 hr., cool, and then pour several small portions (3 to 5 ml.) of alcohol down the condenser to dissolve the residual sodium, followed by 20 to 30 ml. of water. Transfer the contents of the flask to a separating funnel, neutralise to phenolphthalein by means of nitric acid to avoid the formation of an emulsion in the subsequent shaking, separate the water layer, wash the xylene layer several times with water, and determine halide in the combined water layers by Volhard's method. Alternatively, when the solution is highly coloured or contaminated distil off the xylene with steam instead of using a separating funnel.

When, besides halides, other functional groups capable of reacting with sodium are present in the molecule, increase the amount of sodium used correspondingly.

Compounds not amenable to the above-given treatment—With methylene chloride, trichloroethylene, heptachlorotoluene, and 2 : 5-dichloronitrobenzene, use ether initially in place of xylene, boil for 1 to 1·5 hr., then add xylene and boil to complete the reaction. A mixture of ether and xylene at the start or ether alone throughout gives results no better than with xylene alone. The process gives also a less highly coloured product than with xylene alone and facilitates the titration.

In presence of sulphur compounds, *e.g.*, dichlorodiphenylsulphone silver sulphide would appear in the Volhard titration. In such cases precipitate silver chloride and sulphide together, extract the chloride by means of ammonia solution, and re-precipitate the chloride by means of nitric acid, completing the determination gravimetrically.

Determination of chlorine, bromine, and iodine present together—Heat the substance with metallic sodium and xylene as described in general procedure, remove the xylene, and then neutralise the water layer with 20 per cent. sulphuric acid solution. Make up the solution to 250 ml. with water, pipette 50 ml. into a wide 200-ml. beaker, add to it 50 ml. of 10 per cent. magnesium sulphate solution, and 1 to 2 ml. of 20 per cent. sulphuric acid solution, and titrate potentiometrically with 0·1 *N* silver nitrate.

Determination of fluorine—Treat with sodium as for the other halides and determine fluorine by the lead chlorofluoride method. Ethylene fluorhydrin, fluorochloroethane, fluorobenzene, and benzotrifluoride were tested by this method and gave theoretical results. G. S. Smith

Determination of Glycerol in Soap. Rapid Cerate Procedure. L. Silverman (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 410–411)—The perchloratocerate procedure has been applied to the determination of glycerol in soap. Fatty acids, proteins, and chlorides are first removed and an aliquot of the residual solution is treated with perchloric acid and cerate, and the excess of cerate titrated with oxalate solution.

Procedure—Weigh accurately about 20 g. of dry soap, dissolve in 300 ml. of hot water, and add 1 drop of methyl orange indicator (prepared without alcohol). Carefully add 25 per cent. sulphuric acid solution until the liquid is coloured red and add 1 ml. in excess. Maintain the solution at 80° C. until the fatty acids separate on the surface and then cool until the fatty acids solidify. Filter the cold solution, wash the residue with cold water, and discard the fatty acids. To the combined filtrate and washings add, in 1-ml. portions, a 10 per cent. solution of lead acetate previously boiled with lead oxide, until no further precipitation of protein occurs. Dilute the solution to 500 ml., mix well and set aside until the supernatant liquid is clear. Transfer 50 ml. of the solution (2·0 g. of soap) to a beaker and add 25 ml. of water and 25 ml. of 72 per cent. reagent-grade perchloric acid. Add exactly 20 ml. of a solution containing 0·5 *N* ammonium cerate in 6 *N* perchloric acid, heat the mixture to 50° C., and maintain at this temperature for 12 to 13 min., stirring occasionally. Add 100 ml. of cold water, set aside for at least 5 min., and slowly titrate the excess of cerate with 0·2 *N* sodium oxalate, using nitroferroin as indicator. The blue colour disappears about 0·1 ml. short of the end-point and changes to pink at the end-point. The percentage of glycerol in the sample can be calculated from the expression:—

Glycerol per cent. =
[(ml. of cerate as *N*) − (ml. of 0·2 *N* oxalate)]
× 0·0023/weight of sample

[Abstractor's note—Full details of the preparation of ammonium perchloratocerate reagent are given in a free publication to be obtained from The G. F. Smith Chemical Co., Columbus, Ohio.]

A. H. A. Abbott

Polarographic Method of Determining α-Nitronaphthalene in Presence of α-Naphthylamine. Yu. I. Vaynshteyn (*Zavod. Lab.*, 1948, **14**, 517–519)—In a de-oxygenated mixture (1 : 1) of methyl alcohol and water containing potassium chloride in 0·1 *N* concentration as supporting electrolyte and 3 drops of 0·5 per cent. gelatin solution per 10 ml. as maximum suppressor, α-nitronaphthalene gives a well defined polarographic wave with a half-wave potential of −0·26 v. *versus* the normal calomel electrode, and α-naphthylamine gives no wave and has no effect on the wave of the nitro-compound even when its concentration is fifty times that of the latter. The wave-height is strictly proportional to concentration over the range 20 to 200 mg. per litre. In presence of benzene in concentrations up to 2 per cent., similar results are obtained if the alcohol concentration is increased to 80 per cent. Thus, the polarographic method may be used for production control of α-naphthylamine reduced in benzene solution from α-nitronaphthalene. Other nitro-derivatives of naphthalene, *e.g.*, 1 : 8-dinitronaphthalene, behave like α-nitronaphthalene. G. S. Smith

Polarographic Study of Certain Anthraquinones. N. H. Furman and K. G. Stone (*J. Amer. Chem. Soc.*, 1948, **70**, 3055–3061)—The polarographic behaviour of a large number of anthraquinone derivatives has been studied. All the compounds examined give well defined reduction steps suitable for analytical purposes. Buffered solutions of pH 9·0 to 10·0 should be used and the concentration of reducible material should be 1·0 to $2{\cdot}0 \times 10^{-3}\,M$. Under these conditions the half-wave potentials are all about −0·6 to −0·7 v. *versus* the saturated calomel electrode. For the less soluble compounds a solvent containing 40 per cent. by volume of dioxan is used. J. G. Waller

Estimation of Hemicelluloses in Holocellulose from Non-woody Plant Material. E. Bennett (*Anal. Chem.*, 1948, **20**, 642–643)—*Procedure*—To isolate holocellulose from beet, citrus or cranberry pulps, cornstalks, oat straw or mixed hay, extract the sample (ground to pass a 25- but not a 50-mesh sieve) with a mixture of alcohol and benzene (1 : 2) for 6 to 8 hr., and then twice with 0·5 per cent. ammonium citrate solution at 80° C. for about 5 and 19 hr., respectively. After the usual treatment with sodium chlorite and acetic acid, filter off the holocellulose on a cloth, allow it to dry in air, and treat 0·2 g. of it with 20 ml. of 12 per cent. sodium hydroxide solution at 20° C. for 10 min. Add 20 ml. of water at 20° C., and maintain at 20° C. for 90 min., agitating the mixture once during this period. Dilute to 100 ml. in a graduated flask, mix well, filter through glass wool, and boil 25 ml. of the filtrate with 5 ml. of water, 30 ml. of concentrated sulphuric acid, and 5 ml. of 1·0 *N* potassium dichromate for 2 min. at 140° to 150° C., air being passed through slowly to facilitate the boiling. Cool rapidly, and measure the transmittance of the solution in a Coleman Universal Spectrophotometer, with distilled water as standard, and using a wavelength of 600 mμ. and a PC–4 filter. Establish a curve relating glucose and the reduced dichromate by oxidising (as described above) a 25-ml. aliquot of each of 8 solutions containing 0 to 140 mg. of glucose per 100 ml., and plotting the transmission values against the number of milligrams of anhydroglucose used. The curve shows a slight tendency to deviate from Beer's law at the higher concentrations. Report the results on an ash-, moisture- and nitrogen-free basis. In general, agreement within any one set of determinations is better than that between sets of duplicates or triplicates, because the control of the procedure for the isolation of the holocellulose is not so good as that for the subsequent hemicellulose determination. Average values obtained are, for beet pulp 5·9 ± 0·8, citrus pulp 9·2 ± 0·5, cranberry pulp 13·1 ± 0·4, cornstalks 26·5 ± 1·4, oat hay 27·5 ± 1·4, and mixed hay 23·1 ± 1·0 per cent. J. Grant

Replacement of Rochelle Salt by Glycerol in the Determination of Sugar. S. N. Lutokhin and O. A. Kuzmenko (*J. Anal. Chem. Russ.*, 1948, 3, 196–197)—Previous workers who have used glycerol in place of Rochelle salt in Fehling's solution have found that the normal tables could not be used in the higher range of concentrations of sugar. With the composition given below the iodimetric method of Fresenius (*Anleit. z. chem. Anal. d. Weines*, 1922) gives correct results with concentrations not exceeding 5 g. per litre and by using Fresenius's tables.

Procedure—To 10 ml. of the sugar solution add 3 ml. of glycerol, 9 ml. of 20 per cent. potassium hydroxide solution, 10 ml. of 4 per cent. copper sulphate solution, and 10 ml. of water. Then proceed as described in Fresenius's method.

G. S. Smith

Colour Reagents for the Paper Chromatography of Sugars. W. G. C. Forsyth (*Nature*, 1948, **14**, 239–240)—The application of paper chromatography to the identification of sugars has been described (Partridge, *Ibid.*, 1946, **158**, 270). The tests may be made more selective by using resorcinol and naphthoresorcinol as test reagents in addition to the usual ammoniacal silver nitrate reagent.

Procedure—Place three spots of a 1 per cent. solution of the sugar on a filter paper and, alternately, three spots of a standard mixture of sugars, and dry in the usual manner. Cut the paper into three strips and spray each strip, one with ammoniacal silver nitrate solution, one with resorcinol solution, and one with naphthoresorcinol solution. Prepare the phenol solutions by adding 10 ml. of a 1 per cent. solution of the phenol to 90 ml. of 2 *N* hydrochloric acid. Dry the papers sprayed with the phenol solutions at 85° to 95° C. for 10 min. Colours given by the phenols with sugars are—with resorcinol: fructose, red; xylose and arabinose, blue; rhamnose, yellow; with naphthoresorcinol: galactose, glucose, and mannose, grey; fructose, brown; xylose and arabinose, blue; rhamnose, green. Non-reducing sugars also give a positive reaction.

B. Atkinson

Inorganic

New Qualitative Reaction for Boric Acid and its Salts. A. P. Kreshkov and S. S. Vilborg (*J. Anal. Chem. Russ.*, 1948, 3, 172–175)—Boric acid reacts with ethyl orthosilicate or its polymers to give a volatile compound that burns with the characteristic green flame. This reaction is preferable to the normal flame test which requires heating the substance with concentrated sulphuric acid and alcohol and may be dangerous, and to certain colour reactions, *e.g.*, with quinalizarin, etc., since oxidising agents and fluorides do not interfere.

Procedure—Gently heat the dried material under test with a few drops of ethyl orthosilicate or its polymers in a crucible, and apply a burner to the vapour, or heat the mixture in a test tube fitted with an outlet tube. With 3 to 4 drops of the ester the minimum amount of boric acid detectable is 0·6 to 1 mg.

Preparation of ethyl orthosilicate—Place 2·2 g.-mol. of absolute alcohol in a round flask with three necks, carrying a stirrer, reflux condenser, dropping funnel, and capillary for passage of dry air. The condenser is fitted with a calcium chloride tube. Place the flask in a mixture of ice and salt, and introduce through the dropping funnel 0·5 mol. of freshly distilled silicon tetrachloride dropwise with constant stirring. Remove the calcium chloride tube and connect the end of the condenser to a vacuum pump and suck dry air through the reaction mixture for 1 hr. at room temperature and 2 hr. at 80° to 90° C. Then transfer the contents to a Wurtz flask and distil from a glycerol bath at normal pressure.

Preparation of ethyl orthosilicate polymers—To 230 g. of the ester, add, during 2 hr., 100 g. of 80 per cent. by weight ethyl alcohol with constant stirring and cooling. Leave for 2 hr. at room temperature, then heat under a reflux condenser at 100° C. for 2 hr., leave overnight, and finally distil off the alcohol.

G. S. Smith

Colorimetric Determination of Copper by Means of Dimethylglyoxime. V. M. Peshkova, M. E. Levontin, and K. I. Litvin (*J. Anal. Chem. Russ.*, 1948, 3, 161–166)—A study of the colorimetric determination of copper based on the colour obtained with dimethylglyoxime, pyridine, and ammonium persulphate has been made. The method described by Clarke and Jones (*Analyst*, 1926, **54**, 333) is preferable to that of Goethals (*Z. anal. Chem.*, 1936, **104**, 170) since the colour intensity is more stable. The former method may be improved by reducing the amount of sulphuric acid, and also by adding alkali, which gives a more stable colour, though with somewhat reduced sensitivity.

Tests designed to discover if in absence of an oxidant a compound containing both dimethylglyoxime and pyridine, analogous to that found for iron by Chugaev could be obtained, yielded only the dark-lilac compounds $CuSO_4.3{\cdot}5C_5H_5N$ and $CuSO_4.4C_5H_5N$, and the reaction was sensitive to not less than 2 mg. of copper per 100 ml.

Clarke and Jones supposed that the function of the pyridine was to give the necessary alkalinity, but it is most probable that pyridine enters into the composition of the coloured compound. A buffer solution of pH 5 treated with a solution of copper and all the reagents except pyridine remains colourless, but at once assumes the characteristic colour when pyridine is added.

The oxidising agent appears to act not on copper ions, by analogy with the nickel - dimethylglyoxime reaction, but on the dimethylglyoxime. Addition of a nickel salt after various intervals of time showed that the amount of the nickelous-dimethylglyoxime compound that could be precipitated progressively decreased, indicating that oxidation of dimethylglyoxime was taking place.

The most stable colour is obtainable with a 12- to 15-fold excess of dimethylglyoxime.

Procedure—To 50 ml. of the test solution, free from heavy metal ions other than copper and from chlorides, and neutral to litmus, add 1·5 ml. of 0·5 *N* sulphuric acid, 1 g. of dry ammonium persulphate, 1 ml. of 1 per cent. alcoholic dimethylglyoxime solution, 0·5 ml. of 0·5 per cent. silver nitrate solution, 2 ml. of 10 per cent. pyridine solution, and 3·3 ml. of 0·5 *N* sodium hydroxide, adding the reagents in the order given, and the last one gradually with stirring; then dilute to 100 ml., and after 20 to 25 min. compare the colour with a standard treated in exactly the same way at the same time. If no colour appears the amount of copper is less than 0·04 mg. in the 100 ml. In this case repeat the test without the alkali addition, but compare the colour, within 5 min. of adding the reagents, with a standard simultaneously prepared. Alternatively, with 0·05 to 0·6 mg. of copper, the range over which Beer's law is obeyed, use a photo-colorimeter and a calibration curve applicable to the colour intensity obtained after 20 to 25 min., or use two standards and apply Strelkov's formula

$$C_x = C_1 + \frac{(C_2 - C_1)}{(R_2 - R_1)} (R_x - R_1)$$

where C_1 and C_2 are the concentrations of the standard solutions, C_x is the concentration of the test solution, R_1 and R_2 are the drum readings for solutions C_1 and C_2, and R_x is the reading for the test solution.

The method of colorimetric titration suggested for this reaction by Ayzenberg and Menshikova (*Zavod. Lab.*, 1946, **12**, 673) can be used when the amount of copper is less than 0·1 mg. With higher amounts the titration is too prolonged.

Alkali and alkaline earth metals do not interfere with the reaction if a blank consisting of an aqueous solution of the salts present is used. If aluminium and zinc are present copper must be separated first. Any metals that react with the products of oxidation of dimethylglyoxime must be removed.

G. S. Smith

Colorimetric Determination of Small Amounts of Copper in Aluminium. G. I. Ayzenberg and E. M. Menshikova (*Zavod. Lab.*, 1946, **12**, 673–674)—The method described by Clarke and Jones (*Analyst*, 1926, **54**, 333) is modified to allow of a colorimetric titration of small amounts of copper in aluminium.

Procedure—Treat 5 g. of aluminium with 50 to 60 ml. of 20 per cent. sodium hydroxide solution added in small portions. Heat on a sand-bath to complete dissolution, add 20 ml. of 20 per cent. sodium sulphide solution and 350 ml. of water, heat without boiling for 1 hr., and leave overnight. Filter off the precipitate, wash first with 1 per cent. sodium sulphide solution, and then with 5 per cent. sulphuric acid solution to remove zinc. Place the washed precipitate in the original flask, dissolve in 20 to 30 ml. of hot nitric acid, precipitate the sesquioxides with ammonia, filter off the precipitate and wash it, and treat the filtrate with a few drops of perhydrol. Boil to remove coloured products of oxidation of the paper formed by nitric acid, adding more perhydrol and boiling if necessary. Finally, boil to ensure complete decomposition of the perhydrol. Cool, make up the solution to 200 ml. in a graduated flask, transfer 50 ml. to a small flask and just acidify to litmus paper with diluted sulphuric acid (1 + 10). In another similar flask place 50 ml. of water and acidify with 1 drop of acid. To each flask add 2 ml. of 0·5 per cent. silver nitrate solution, 1 ml. of 10 per cent. pyridine solution, 1 ml. of saturated alcoholic dimethylglyoxime solution, and 0·5 g. of ammonium persulphate. If copper is present in the test solution a raspberry colour appears. To the contents of the second flask, add dropwise from a microburette a copper sulphate solution containing 0·1 mg. of copper in 1 ml. until the solutions are of the same colour.

The method is most suitable for the determination of 0·01 to 0·1 mg. of copper in the 50-ml. aliquot portion.

G. S. Smith

Precipitation of Mono- and Di-Zirconyl Phosphate and the Use of Zirconyl Salts for the Separation and Determination of Phosphoric Acid. R. Stumper and P. Mettelock (*Bull. Soc. Chim.*, 1947, 674–676 M)—Phosphoric acid is precipitated quantitatively from a hydrochloric acid solution of a simple salt or of a slag by adding an excess of zirconium oxychloride solution. The precipitate forms dizirconyl phosphate, $2ZrO_2.P_2O_5$, on ignition. Monozirconyl phosphate is obtained if phosphoric acid is in excess, or if precipitation is carried out in sulphuric acid solution. The dizirconyl phosphate is less soluble and less readily hydrolysed than is the monophosphate. The procedure given below can be used for determining phosphate or for removing phosphate before the precipitation of the third group of metals in qualitative analysis.

Procedure—Prepare a solution of the phosphate containing about 0·1 g. of phosphorus pentoxide in a mixture of about 40 ml. of water and 20 ml. of hydrochloric acid (d. 1·07). Add a 200 per cent. excess of a 10 per cent. solution of zirconyl chloride, evaporate to dryness, and digest the residue at 130° C. with 20 ml. of hydrochloric acid (d. 1·07) for 1 hr. Filter the liquid and wash the precipitate with warm, diluted hydrochloric acid (1 + 10). Ignite the precipitate at 1050° C. for 1 hr. and weigh as $2ZrO_2.P_2O_5$. The method has been used for determining phosphorus in slags and in steels.

B. Atkinson

Micro-Determination of Bromides and Iodides in Presence of Chlorides. I. Bellucci (*Gazz. Chim. Ital.*, 1942, **72**, 501–507)—The method is intended for the determination of iodine and bromine in blood and other biological material. The organic matter is destroyed by careful incineration in presence of sodium hydroxide according to the process previously described by the author (*Analyst*, 1935, **60**, 263), the determination being carried out on the resultant ash.

Procedure—Extract the ash with 95 per cent. alcohol, thereby dissolving part of the chloride and the whole of the bromide and iodide present. Evaporate the solution to dryness and re-dissolve the residue in 15 to 20 ml. of water. Place the solution in a 50-ml. separating funnel and add a few drops of diluted sulphuric acid (1 : 10) and 3 to 4 ml. of carbon tetrachloride, followed by 2 ml. of a 1 per cent. solution of sodium nitrite. Separate the tetrachloride layer containing the liberated iodine and extract the aqueous layer three or four times with further small quantities of tetrachloride. To the total extract add, drop by drop with continuous shaking, 10 per cent. sodium hydroxide solution until the colour is completely discharged, and evaporate to dryness on the water-bath. Re-dissolve in about 10 ml. of water, add a crystal of potassium permanganate, and heat to incipient boiling. Destroy the excess of permanganate with a few drops of alcohol, filter on a small filter, and wash with boiling water, keeping the volume as small as possible. Evaporate to about 10 ml. Remove any excess of nitrous acid by adding 0·1 g. of urea and 10 drops of acetic acid and boiling for 3 to 4 min. Cool, add a few centigrams of sodium iodide and 2 to 3 drops of dilute sulphuric acid, and titrate the liberated iodine with 0·002 *N* thiosulphate.

Make the aqueous solution containing the chloride and bromide slightly alkaline by adding sodium hydroxide, and evaporate to dryness on the water-bath. Re-dissolve in 20 to 25 ml. of water and transfer to a small flask. Add 0·5 g. of pure sodium chloride, 5 ml. of *N* sodium hydroxide, 30 ml. of freshly prepared chlorine water containing not less than 5 mg. of chlorine per ml. and 1 g. of boric acid. Heat for 10 min. over boiling water to oxidise the bromide to bromate. To the hot liquid add 2 ml. of 10 per cent. sodium formate solution and boil on an asbestos-covered gauze to eliminate the excess of hypochlorite, continuing until the volume is reduced to about 15 ml. Cool, add 3 drops of 5 per cent. potassium iodide solution, 2 ml. of starch solution, 1 drop of 5 per cent. sodium molybdate, and 3 ml. of 2 *N* hydrochloric acid. After 4 to 5 min. standing, sheltered from light, titrate with 0·004 *N* thiosulphate. Make blank tests on the reagents.

A. H. BENNETT

Co-precipitation of Uranous Sulphate with Rare-Earth Double Sulphates. A. W. Wylie (*Nature*, **1947**, **160**, 830)—Uranous ions in acid solution are co-precipitated with sodium cerium sulphate and the sodium rare-earth sulphates. Almost complete removal of uranous ions from solution is obtained by treating an acid solution containing not less than 30 g. per litre of rare-earth oxides and about 3 g. per litre of uranous oxide with sufficient sodium sulphate to precipitate the rare earths completely. Uranyl ions are not co-precipitated under these conditions.

B. ATKINSON

New Dry Test for Gold. R. C. Mehrotra (*Nature*, 1948, **161**, 321)—To the gold salt in a porcelain dish add a quantity of concentrated hydrochloric acid and a speck of zinc. Insert a test tube full of water into the solution over the point where the hydrogen is bubbling up. Hold the test tube in the hottest part of a bunsen flame. If gold is present a brilliant green mantle forms round the bottom of the test tube. The test is sensitive to 0·1 mg. of gold in 1 ml. of hydrochloric acid. Copper interferes and, in presence of tin, the gold green mantle is replaced by the blue mantle given by tin.

B. ATKINSON

Improvement of Analytical Control for Silica Brick. J. T. Rozsa (*J. Amer. Ceram. Soc.*, 1948, **31**, 280–283)—In general, the preparation of a representative sample of silica bricks is difficult. The author takes 20 to 50 bricks from a car-load, chips a small portion from each and grinds the composite sample, finally sampling in the usual way. After mixing with an equal weight of pure carbon dust, the sample is completely consumed in a D.C. arc, placed 110 cm. from the slit of the spectrograph to minimise the effect of wandering of the discharge. Standards of the same approximate composition as the sample to be analysed are photographed on each plate and allow the oxides Al_2O_3, MgO, Fe_2O_3, CaO, Na_2O, TiO_2, K_2O, ZrO_2, MnO_2, and Li_2O to be estimated. As the silica content is fairly constant this material is used as an internal standard in preference to an added material, such as copper oxide. Using the method as described, an accuracy to within ±5 per cent. of the amount of minor constituent is obtainable in routine practice. The time of analysis is about 1 hr., 40 min. of which are taken in preparing the sample.

H. R. CLAYTON

Calculation of the Content of Volatile Matter in Coal to Ash- and Water-free Coal. J. Hamaker (*Chem. Weekblad*, 1948, **44**, 517–525)—In computing the content of volatile matter in coal to an ash- and water-free basis there are considerable uncertainties. The loss in weight resulting from decomposition of the mineral matter is unknown owing to uncertainty in the water content of the shale and in the reactions that occur. It is possible to separate the coal fraction from a powdered coal by treatment with a mixture of benzene and carbon tetrachloride of density 1·4, and the content of volatile matter can then be determined on this fraction. This method is quicker than the indirect one. That the treatment does not fractionate the coal is shown by the constancy in calorific value and organic sulphur content, when calculated on dry mineral-free basis. This separation is necessary when the content of volatile matter is used as a basis for classifying coal, and it should be used for all coals high in carbonate. The A.S.T.M. Designation D388–38 does not take into account the volatile matter resulting from decomposition of the mineral matter.

G. MIDDLETON

Physical Methods, Apparatus, etc.

Oscillographic Polarography with Periodical Triangular Voltage. A. Sevcik (*Coll. Czech. Chem. Comm.*, 1948, **13**, 349–377)—An equilateral triangular, periodical voltage is used to charge and discharge the dropping mercury electrode linearly with time, and current - voltage curves are recorded with a cathode-ray oscillograph.

The reduction processes for cadmium, plumbic, and thallous ions have been shown to follow the equations calculated for reversible reactions. The maximum of the electrolytic current is proportional to concentration in solutions that are less than $10^{-3}\,N$ with respect to the reducible ions.

J. G. WALLER

Application of Corrections in Viscometry of High-Polymer Solutions. R. H. Wagner (*Anal. Chem.*, 1948, **20**, 155–157)—This paper discusses the importance and magnitude of corrections for density and kinetic energy in the determination of relative and hence of intrinsic viscosities.

If t/t_0 is the ratio of the efflux times of a solution and pure solvent of densities d and d_0, respectively, the true relative viscosity η_r is given by—

$$\eta_r = \frac{dt}{d_0 t_0} + \frac{mVd}{8\pi l \eta_0 t} \times \left[\left(\frac{t}{t_0}\right)^2 - 1\right].$$

Putting $d/d_0 = \beta$, and $t/t_0 = \alpha$, and $\dfrac{Vd_0}{8\pi l \eta_0 t_0} = \mathrm{K}$, we may write $\eta_r = \alpha\beta + \beta\mathrm{K} \times \dfrac{\alpha^2 - 1}{\alpha}$, where V is the volume of the measuring bulb, l the capillary length, and m a coefficient, here assumed to be unity. If C is the concentration of the solution in grams per 100 ml., then the Inherent Viscosity $\{\eta\} \equiv \dfrac{\log_e \eta_r}{C}$, which should not be confused with the Intrinsic Viscosity $[\eta] \equiv \underset{C \to 0}{\mathrm{Lt}}\{\eta\}$. The inherent viscosity is a function of the concentration, whereas the intrinsic viscosity, obtained by graphical extrapolation of the inherent viscosity to $C = 0$, is not.

Contribution of the density factor—The error produced in the inherent viscosity $\{\eta\}$ due to the departure of β from unity ($\Delta\beta$) is given by $\Delta\beta/C$. This correcting term may be ignored if the allowable uncertainty in $\{\eta\}$ is 0·01 unit or more.

Contribution of the kinetic energy factor—The correcting term due to the kinetic energy is given by $\Delta\{\eta\}_{\mathrm{kc}} = \dfrac{\mathrm{K}(\alpha^2 - 1)}{\alpha^2 C}$ if second magnitudes are ignored. A nomogram is given to facilitate calculation of this correcting factor or to establish conditions such as solvent density and viscometer constants necessary to keep the correction within specified limits. The fully corrected value of the inherent viscosity is

$$\{\eta\} = \frac{\log_e \alpha}{C} + \frac{\Delta\beta}{C} + \frac{\mathrm{K}(\alpha^2 - 1)}{\alpha^2 C}$$

W. C. WAKE

Capillary Tube Viscometer for Routine Measurements of Dilute High-Polymer Solutions. R. H. Wagner and J. Russell (*Anal. Chem.*, 1948, **20**, 151–155)—The viscometer is particularly suited for routine measurements of dilute high-polymer solutions such as are required for determining molecular weights from the Staudinger equation.

Apparatus—The complete apparatus is shown in Fig. 1, and comprises a viscometer, A, and a thermometer and thermometer jacket, B. The viscometer itself is shown in Fig. 2, and consists of an inner and outer assembly, the former having a measuring bulb, J, and capillary, M, of 0·5-mm.

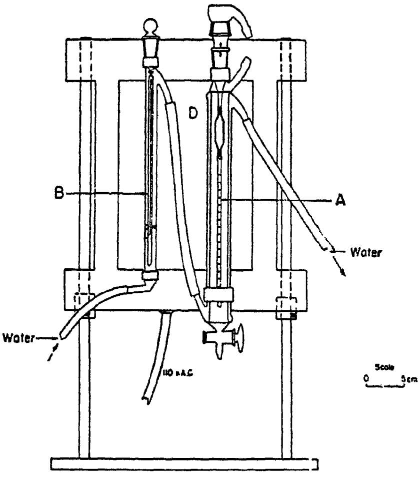


Fig. 1

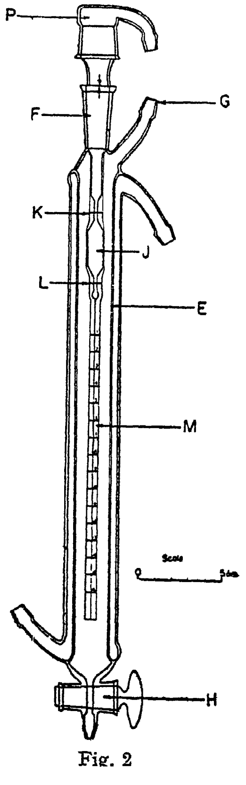


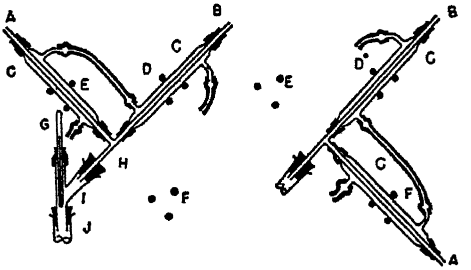
Fig. 2

precision-bore tubing of 190-mm. length. The bulb has a volume (between marks K and L) of about 1 ml. From 3 cm. below the lower transit mark, filling marks are engraved on the capillary at 16 intervals of 1 cm. These enable different average internal heads of liquid to be used and, consequently, the outflow time to be adjusted.

Procedure—Pour sufficient liquid into the outside jacket to come above the desired filling mark. Insert the inner assembly, and, after allowing time for thermal equilibration and drainage, adjust accurately to the filling mark. Fill the measuring bulb, J, by application of pressure at G and obtain the time for outflow of the volume of liquid contained between the transit marks. It is convenient to determine outflow times at various filling levels for solvents to be used, and, apart from occasional checks, these may be taken as standard, only the polymer solution viscosities being normally measured. The relative viscosity is assumed to be the ratio of the outflow times of the solution and solvent. The density and kinetic energy corrections are small and may usually be ignored. Instructions for calculating these corrections, together with a nomogram, are given for use where great accuracy is desired (see preceding abstract, Wagner, *Ibid.*, 155–157). W. C. Wake

Simple Still-Head. R. W. Hakala (*J. Chem. Educ.*, 1948, **25**, 465)—The apparatus, which has no stopcock, is kept in the reflux position during operation until the vapours reach a constant temperature when the fraction is collected by rotating the reflux arm into the "take-off" position until the temperature again begins to rise, when the arm is returned to the reflux position.

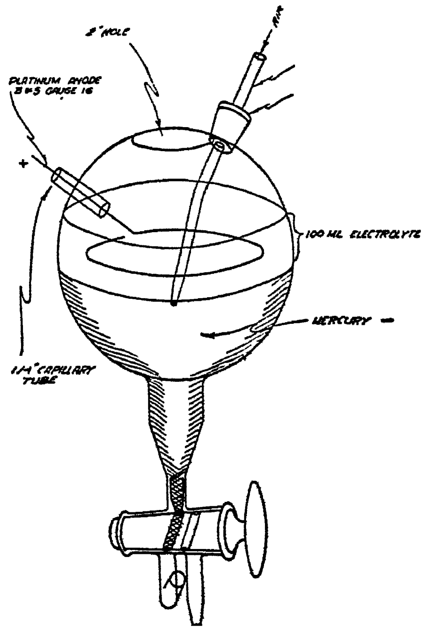
M. E. Dalziel



Reflux position. Take-off position.

A. Angular reflux arm.
B. Stationary reflux arm.
C. Micro Liebig condenser jacket (used when necessary).
D. Clamp.
E. Clamp for A in reflux position.
F. Clamp for A in take-off position.
G. Thermometer.
H. Stopper to hold T-tube; with hole large enough to allow rotation of T-tube, but snug enough to be gas-tight; lubricated with glycerol or stopcock grease.
I. Addition tube.
J. Fractionating column.

Mercury Cathode Cell for Rapid Electrolysis. F. T. Rabbitts (*Anal. Chem.*, 1948, **20**, 181–182)—The cell is made from a 700-ml. Pyrex Florence flask modified as shown in the figure. The platinum wire anode has a diameter of 10 cm. and lies horizontally about 0·5 cm. above the mercury surface. The air-inlet tube through which a slow current of air is passed dips 1 cm. below the centre of the mercury surface. A rubber stopper fitting into the 2-in. hole at the top of the cell carries a small U-tube designed to contain 5 or 6 ml. of distilled water to remove spray from the gases leaving the flask. A 300-ml. flask is used as a mercury levelling bottle. Electric contact with the mercury is made by a length of platinum wire held in place by a rubber stopper containing an air vent. A current supply of up to 7 amp. at 6 v. is required.



Procedure—Electrolysis is usually performed in 0·3 *N* sulphuric acid solution. Electrolyse for 30 to 40 min. with a current of not less than 6 amp. Lower the levelling bottle and, as soon as the mercury has drained from the cell, run the solution into a 400-ml. beaker. Rinse the spray trap and add the washings to the beaker. If the solution contains a suspension, add filter pulp and filter the liquid through a No. 30 Whatman paper. Add 30 ml. of 2 per cent. sulphuric acid solution to the cell, raise the levelling bottle, and continue electrolysis for 5 min. Run off the wash liquid, rinse the spray trap again, and filter the combined washings through the original paper. B. Atkinson

Review

INORGANIC CHEMISTRY. FRITZ EPHRAIM. Fifth English Edition. Revised. By P. C. L. THORNE, M.A., M.Sc., Ph.D., F.R.I.C., and E. R. ROBERTS, A.R.C.S., Ph.D., D.I.C. Pp. xii + 939. London: Gurney & Jackson. 1948. Price 32s.

The four previous English editions of Fritz Ephraim's well-known book were favourably reviewed in *The Analyst* (1926, **51**, 651; 1934, **59**, 309; 1940, **65**, 584; 1944, **69**, 139). In these reviews, written by able chemists and experienced analysts, will be found all that can be said about this book; indeed, to paraphrase the suggestion of one writer, who thought that the only adequate method of reviewing it would be to issue an order that it should be read by all chemists, one might, with some excuse, suggest that anyone wishing for a considered opinion on the fifth edition should look up the reviews of the previous four and not trouble to read this review any further; but as two of the previous reviews appeared in what are now becoming scarce war-time issues of *The Analyst*, there is perhaps some excuse for writing another, even at the risk of incurring a charge of plagiarism.

The outstanding feature of the book is the arrangement of the subject-matter; this has been done as though a full-scale modern textbook of advanced inorganic chemistry of the classical type, which treats each element and its compounds under a separate heading, had been cut loose from its binding, had had all the pages carrying information of a chemically related nature sorted into separate groups and then, with the additional matter necessary to make a continued story, had been rebound into a new volume. There is, of course, rather more to it than that. There is the critical acumen that has gone to the collection and selection of material for inclusion, the clarity of the style in which it is written and the quality of the typography, all of which make for ease in the reading of a subject that is, in many parts, by no means too easy. Nevertheless, the value of the book and its absorbing interest to the reader lie very largely in this ingenious grouping of related chemical facts and information. In pursuing this method of arrangement the author takes the same cross-sectional view of chemistry that an examiner so often takes in setting a certain type of question; for instance, the answer to a question such as, "Exemplify and account for the various methods that are used in the extraction of metals from their ores," will, in "Ephraim," be found complete in one place, instead of scattered over several hundred pages as it is in the classical inorganic texts. This method of imparting collateral information in juxtaposition will appeal to students working for the higher examinations in chemistry; it also makes a very readable book for those to whom examinations are no longer of pressing importance, but who may wish to keep in touch with modern thought in inorganic chemistry; for it enables the reader to review the existing collection of chemical facts and theories as a whole and to appreciate the interconnection and relative importance of the various parts. Modernity is the key-note of the book; its chemistry begins with the Rutherford - Bohr atom and the co-ordination theory; in it there is to be found neither history nor human interest. This must surely be the only book on general inorganic chemistry in the English language that contains no mention of John Dalton. In this strict adherence to a set plan much that is of interest is lost, but the gain is great and more would certainly have been lost had the inclusion of other matter been allowed to interfere with the consistent scheme of the book.

The revision of this fifth edition has been thorough; there is evidence that consideration has been given to errors noted in previous reviews, but an implication that copper is not precipitated in an alkaline tartrate solution by ammonium sulphide remains, on page 469, to surprise an analyst. F. L. OKELL

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

The Use of the Plate Method for the Assay of Aneurine in Yeast and Yeast Products

BY A. JONES AND S. MORRIS

(Read at the Annual General Meeting of the Biological Methods Group, on December 17th, 1948)

THE assay of aneurine, both by the chemical and microbiological methods, has in the past yielded results of very doubtful accuracy. Of the chemical methods the thiochrome technique is, perhaps, the simplest and most frequently used. With pure aneurine solutions this method yields satisfactory results, but in presence of other materials, as in the assay of foodstuffs, great difficulty is found in the extraction of the aneurine uncontaminated with various coloured compounds which interfere markedly with the technique of assay.

Various microbiological methods have, at different times, been published, the yeast fermentation method of Schultz *et al.*,[1] the mould technique using *Phycomyces blakesleeanus*[2,3] and the bacteriological methods using *Streptococcus salivarius*[4] and *Lactobacillus fermentum*.[5] There are disadvantages in each of the methods, such as the special apparatus necessary for the yeast fermentation, the time required, two weeks, for the growth of the mould *Phycomyces* and the inherent difficulties in turbidimetric methods used with *L. fermentum*. Further, most microbiological techniques suffer from the disadvantage that the organisms respond to both the pyrimidine and the thiazole moieties of the aneurine molecule and allowance must be made for these factors.

Recently, a plate method of assay, using *L. fermentum*, has been published by Bacharach and Cuthbertson.[6] The method is not particularly sensitive but would appear to eliminate, at least in part, some of the difficulties encountered in the turbidimetric method. Further, the plate method is rapid and requires no special apparatus.

The present paper details a study of this method and gives a modified technique for the assay of aneurine in yeasts and yeast extracts. The final method adopted is given first, followed by experimental data on which the method is based. This has been done for ease of reading and of subsequent reference.

METHOD OF ASSAY

BASAL MEDIUM—

The basal medium used was that advocated by Sarett and Cheldelin[5] with addition of sodium chloride and 1·5 per cent. of agar. The composition of this medium is shown in the table overleaf.

This was prepared in lots of at least 2000 ml., tubed in 20-ml. amounts, autoclaved at 10-lb. pressure for 10 minutes, and stored at laboratory temperature until required. More accurate results with fewer irregularities in the readings were obtained when the medium was stored for at least 3 days after autoclaving, before being used. Normally, the medium was used within 2 weeks of preparation, but satisfactory results have been obtained with a medium stored as long as 5 weeks.

STOCK CULTURES—

The organism used, *Lactobacillus fermentum* P.36, was maintained on the medium of Cheldelin, Bennett and Kornberg,[8] and sub-cultured every 3 weeks. This medium contained 1 per cent. of Difco yeast extract, 1 per cent. of glucose and 2 per cent. of agar, with addition of 10 μg. of aneurine per tube.

Glucose	20·0 g.
Sodium acetate (anhydrous)	6·0 „
Sodium chloride	5·0 „
Casein hydrolysate	2·5 „
Adenine	0·01 „
Guanine	0·01 „
Uracil	0·01 „
L-Cystine	0·1 „
Peptone (photolysed)	200 ml.
Inorganic salt solution A[7]	5·0 „
Inorganic salt solution B[7]	5·0 „
Riboflavine	100 μg.
Biotin	0·4 „
Pyridoxine	100 „
Calcium pantothenate	100 „
Nicotinic acid	100 „
p-Amino benzoic acid	100 „
Folic acid	1·0 „
Volume made up to	1000 ml. at pH 6·5

PREPARATION OF THE INOCULUM—

L. fermentum was grown for 18 hours at 37° C. in 10 ml. of basal medium minus agar, with the addition of 10 μg. of aneurine per ml. The medium was centrifuged, the supernatant liquor discarded and the organisms washed twice with saline. The washed organisms were finally suspended in 20 ml. of sterile saline and used as inoculum.

PREPARATION OF THE PLATES—

Five tubes of medium were used for the standard and for each test sample. The contents of the tubes were melted by immersing the tubes in a boiling water-bath. The tubes were cooled to a temperature of 48° to 50° C. and maintained at that temperature, and 1 ml. of the inoculum was added to each tube. Immediately after addition of the inoculum the contents of each tube were thoroughly mixed by rotation to distribute the bacterial suspension evenly throughout the medium. The contents of the tubes were poured severally into sterile Petri plates, 9 cm. diameter, on a flat, even surface, allowed to set, dried for 1·5 hours at 37° C. with the lids of the plates raised about half an inch from the bottom of the dishes on a specially constructed drying rack, and finally stored at laboratory temperature until used, usually 2 to 3 hours later.

Five holes were cut in each plate, each hole 10 mm. in diameter, the agar discs removed and the holes sealed with a drop of melted medium.

PREPARATION OF THE YEAST SAMPLES—

1 g. of the dried yeast and 0·05 g. of takadiastase were added to 15 ml. of 1 per cent. sodium acetate buffer at pH 4·5. The pH of the mixture was adjusted to 4·4 to 4·5 with *N* acetic acid or sodium hydroxide as necessary, 2 drops of benzene were added, and the whole was incubated at 37° C. for 16 to 18 hours.

It was then steamed for 10 minutes, cooled, adjusted to pH 6·5, diluted to 25 ml. with water and filtered. This filtrate was diluted 1 : 2, 1 : 4, 1 : 8 and 1 : 16. 0·1 ml. of the 1 : 16 dilution was measured into the first hole of each of the five plates, 0·1 ml. of the 1 : 8 dilution into the second hole of each of five plates and so on, 0·1 ml. of the undiluted filtrate being measured into the fifth holes.

PREPARATION OF THE STANDARD CURVE—

The assay range used for the preparation of the standard curve was 0·25, 0·50, 1·00, 2·00 and 4·00 μg. of aneurine hydrochloride per ml. 0·1 ml. of the aneurine standard solution containing 0·25 µg. per ml. was measured into the first hole of each of five plates, 0·1 ml. containing 0·50 µg. per ml. into the second hole of each plate, and so on. The plates were incubated for 18 hours at 37° C. with the lids raised.

The diameters of the zones of growth were measured to the nearest 0·5 mm. and the mean values plotted against the logarithms of the concentrations of the standard aneurine hydrochloride in µg. per ml. or against the logarithms of the concentrations of the yeast samples.

Under the given test conditions, where the effect of doubling the aneurine concentration was to increase the zone diameters by 2·0 mm., the following equation expresses this relationship: $\log D = aZ - b$, where a and b are constants, D is the aneurine concentration in µg. per ml. and Z the zone diameter in mm. corresponding to a yeast concentration of M mg./ml. With the given test conditions, $a = 0{\cdot}1505$ and b is dependant upon the intercept of the standard curve for any given day. From this, the aneurine concentration in the yeast in µg. per g. is given by 1000 D/M.

EXPERIMENTAL

1. EFFECT OF DENSITY OF BACTERIAL SUSPENSION ON ZONE DIAMETERS—

The density of the inoculum used affected the zone diameters markedly. Typical diameters obtained with the bacterial suspension diluted to 20 ml., to 50 ml. and to 100 ml. are given in Table I as the mean of the five zones. The range of the five individual observations was never more than 1 mm.

The zones of growth obtained diminished in sharpness and increased in size as the dilution of the suspension increased.

TABLE I

EFFECT OF DILUTING THE BACTERIAL SUSPENSION ON THE SIZE OF THE ZONE DIAMETERS

Dilution of bacterial suspension	Mean zone diameter in mm. 0·25 µg. Aneurine	0·50 µg. Aneurine	1·0 µg. Aneurine	2·0 µg. Aneurine	4·0 µg. Aneurine
In 20 ml.	18·7	20·4	22·3	24·2	25·9
In 50 ml.	19·3	21·2	23·4	25·3	27·0
In 100 ml.	22·3	24·9	27·1	28·8	31·4

With a bacterial suspension diluted to 20 ml., the mean value of fifteen assays of a yeast was 92·7 µg. per g. of yeast; standard error of the mean, 1·59. With a bacterial suspension diluted to 50 ml., the mean value of fifteen assays was 83·7 µg. per g.; standard error of the mean, 2·93. Further reference is made to these figures at a later stage.

Because of the clarity of the zones and the ease of measurement, the bacterial suspension was in all cases diluted to 20 ml. as the most suitable volume.

2. EFFECT OF PRELIMINARY DRYING OF THE PLATES—

A comparison was made of the zones of growth obtained when the plates were poured and used directly for assay and when the plates, after pouring, were given a preliminary drying of 1·5 hours at 37° C. When the plates were used directly the zones were 4 to 6 mm. larger in diameter, more diffuse and difficult to measure as compared with those that had been subject to a preliminary drying. The preliminary drying at 37° C. for 1·5 hours was therefore included in the standard procedure.

3. EFFECT OF THE pH OF THE STANDARD ANEURINE SOLUTION ON THE ZONE DIAMETERS—

Standard aneurine solutions were prepared using distilled water at pH 6·5, buffer solution at pH 4·5 and buffer solution at pH 7·0. The average zone diameters obtained with these solutions are shown in Table II.

TABLE II

EFFECT OF pH OF THE STANDARD ANEURINE SOLUTION ON THE SIZE OF THE ZONE DIAMETERS

Standard aneurine solution	Mean zone diameter in mm. 0·25 µg. Aneurine	0·50 µg. Aneurine	1·0 µg. Aneurine	2·0 µg. Aneurine	4·0 µg. Aneurine
Distilled water ..	19·1	21·5	24·1	26·5	28·6
Buffer solution, pH 4·5	19·2	21·5	24·3	26·4	28·5
Buffer solution, pH 7·0	19·0	21·4	23·7	25·4	27·5

In solutions with a pH not greater than 6·5 there would appear to be little or no difference, irrespective of the pH, in the zone diameters. In neutral solution, however, variations occurred, the zone diameters at the higher concentrations of aneurine being less than those found in acid solution. For this reason the aneurine solution was never maintained at a pH greater than 6·5.

4. EFFECT OF TAKADIASTASE AND PAPAIN ON ANEURINE—

Enzymatic digestion of foods with takadiastase and papain has been recommended for the complete extraction of aneurine and the conversion of the co-carboxylase to aneurine.[5,9] There appear to be no published results, however, on the effect of takadiastase and papain on aneurine itself. For this reason experiments were carried out on the effect of these enzymes on solutions of pure aneurine.

A solution containing 50 μg. of aneurine hydrochloride per ml., in acetate buffer at pH 4·5, was treated in the following manner:—

(*a*) 20 ml. were incubated at 37° C. for 18 hours with 0·05 g. of takadiastase.
(*b*) 20 ml. were incubated at 37° C. for 18 hours with 0·05 g. of takadiastase and 0·05 g. of papain.

To both tubes 2 drops of benzene were added before incubating. After incubation the tubes were steamed for 10 minutes and cooled, the pH was adjusted to 6·5 and both were diluted to 200 ml. with water. On assay, the results obtained were 5·2, 5·1 and 3·7, 3·2 μg. of aneurine per ml. respectively, the theoretical value being 5·0 μg. per ml.

It would appear that the presence of papain caused a loss of aneurine, whereas takadiastase had no effect. It was, therefore, decided to omit papain from the enzyme mixture added to extract the aneurine from yeast.

On purely theoretical grounds there would appear to be little reason for the addition of papain together with takadiastase for the extraction of aneurine, since the takadiastase contains a protease active in an acid medium and a protease would appear to be the only active enzyme in papain. The reason for the loss of aneurine in presence of papain is difficult to understand and calls for further work.

5. EXTRACTION OF ANEURINE FROM YEAST—

Although takadiastase had no effect on solutions of pure aneurine, it was necessary to ascertain if takadiastase digestion of a material such as yeast would be sufficient to extract all the aneurine present. A series of experiments was, therefore, carried out in which a direct digestion of yeast with takadiastase was compared with the effect of 0·1 *N* sulphuric acid and with that of sulphuric acid followed by takadiastase treatment.

To ascertain in the first place the amount of takadiastase necessary for maximum extraction of the aneurine, different amounts were added to a constant amount of dried yeast or yeast extract. The mixtures were incubated in acetate buffer of pH 4·5 at 37° C. for 18 hours. The assay results are given in Table III.

TABLE III

EFFECT OF DIFFERENT AMOUNTS OF TAKADIASTASE ON THE EXTRACTION OF ANEURINE FROM YEAST

	μg. Aneurine per g. of material	
Amount of takadiastase added	Assay (i)	Assay (ii)
1 g. Dried yeast A + 0·01 g. takadiastase	94	59
1 g. " " + 0·05 g. "	89	97
0·1 g. Yeast extract A + 0·05 g. takadiastase	512	542
0·1 g. " " + 0·10 g. "	532	
0·1 g. " " + 0·15 g. "	450	
0·1 g. Yeast extract B + 0·01 g. takadiastase	210	
0·1 g. " " + 0·05 g. "	445	

The results indicate that 0·05 g. of takadiastase is sufficient to release the aneurine from 1 g. of dried yeast or 0·1 g. of the yeast extracts assayed.

For the comparative trials, using 0·1 *N* sulphuric acid and takadiastase, 0·05 g. of takadiastase per 1 g. of dried yeast was used. The treatment with the acid consisted of steaming 1·0 g. of dried yeast with 10 ml. of 0·1 *N* acid for 30 to 45 minutes. 10 ml. of acetate buffer of pH 4·5 were then added, the pH was finally adjusted to 6·5 and the volume brought to 25 ml. with water. In those experiments in which takadiastase digestion followed acid treatment, the mixture, after steaming, was treated with 10 ml. of acetate buffer and 0·05 g. of takadiastase, and adjusted to pH 4·5, 2 drops of benzene were added, and the whole was incubated for 18 hours at 37° C. The results of the assays are given in Table IV.

TABLE IV

COMPARISON OF THE EFFECTS OF 0·1 *N* SULPHURIC ACID, 0·1 *N* SULPHURIC ACID FOLLOWED BY TAKADIASTASE, AND TAKADIASTASE ALONE ON THE EXTRACTION OF ANEURINE FROM YEAST

Treatment of yeast	μg. Aneurine per g. of yeast	
	Yeast A	Yeast B
0·05 g. Takadiastase alone	94, 99	69
0·1 *N* H_2SO_4—steamed 30 minutes	95	58
0·1 *N* H_2SO_4—steamed 30 minutes and followed by takadiastase	90	67
0·1 *N* H_2SO_4—steamed 45 minutes	67	—
0·1 *N* H_2SO_4—steamed 45 minutes and followed by takadiastase	96	—

Steaming in presence of acid does not always appear to extract all the aneurine from yeast, for subsequent treatment with takadiastase sometimes yielded a higher result. On the other hand, takadiastase treatment alone appeared to be as effective as steaming followed by enzyme treatment. Using the method of enzyme treatment alone, a series of assays was carried out on two samples of dried yeast. Fifteen assay values were obtained with one yeast and eighteen with the second. The results are given in Table V, which shows the mean values and the standard errors.

TABLE V

THE ASSAY OF TWO DRIED YEASTS GIVING THE MEAN VALUE AND THE STANDARD ERROR OF THE MEAN

	Yeast A μg./g.	Yeast B μg./g.
	97, 99, 94, 89, 93, 95, 82, 84, 89, 99, 99, 84, 89, 99, 99	67, 68, 71, 71, 70, 70, 70, 66, 69, 66, 67, 61, 61, 60, 62, 71, 71, 69
Mean value	92·7	67·2
Standard error S_n of the mean	1·59	0·90

6. EFFECT OF AN ANEURINE-FREE YEAST EXTRACT ON THE ASSAY OF PURE ANEURINE—

The possibility existed that the test samples, after treatment, contained some factor or factors affecting the assay. These would not be present in the standard aneurine solution and might affect the final results. This was examined by preparing an aneurine-free yeast extract, by autoclaving in presence of sodium hydroxide. The alkali-treated yeast extract was adjusted to pH 6·5 and, after filtration, diluted as for a normal yeast sample. Dilutions of the aneurine standard solution were prepared with this aneurine-free extract, by dissolving known amounts of aneurine in it. It is realised that this treatment is drastic and may possibly destroy some other unknown essential factor, but any method involving the destruction of aneurine must be similarly suspect.

Examination of the plates and measurement of the zone diameters showed clearly that yeast treated with sodium hydroxide to render it free from aneurine did not contain any factor that would affect the assay of aneurine. It was concluded that, for the present and until further work is carried out, yeast may be assumed to contain no substances which interfere with the assay of aneurine by the method adopted.

Since sending to the press, the effects of pyrimidine and thiazole, separately and together at levels equivalent to the aneurine content of yeast, have been examined. In no case was any growth of *L. fermentum* noted. It can, therefore, be assumed that, in the plate method, these compounds do not affect the assay results.

7. RECOVERY OF ANEURINE WHEN ADDED TO YEAST—

Proof of the validity of the method rested on the ability of the assay technique to give theoretical results for aneurine added to yeast. This was tested by adding different quantities of aneurine, 20, 40 and 100 μg. per g. of yeast, to a yeast sample or to a yeast extract, incubating with takadiastase for 18 hours at 37° C. and assaying as previously described.

The results are given in Table VI.

TABLE VI

RECOVERY OF ANEURINE ADDED TO YEAST AND YEAST EXTRACTS

μg. Aneurine per g. of yeast	μg. Aneurine added per g. of yeast	Total μg. aneurine present	μg. Aneurine found per g.	Per cent. found
82	20	102	108	106
82	40	122	110	90
108	100	208	204	98

μg. Aneurine per ml. yeast extract	μg. Aneurine added per ml.	Total μg. aneurine present	μg. Aneurine found per ml.	Per cent. found
3·2	2·0	5·2	5·1	98

The recovery of the added aneurine was satisfactory and it was considered that the validity of the method, at least for yeast and yeast extracts, had been proved.

DISCUSSION

The plate assay method appears to give more reproducible results than any of the previous methods, although its sensitivity is not particularly good. This one might expect from a plate assay method.

The dilution of the final inoculum is important; the greater the dilution the more vague and uncertain the final zone of growth. For this reason a dilution of 1 in 20 has been adopted in the final method. Although the zones of growth are small they are well defined and easily measured. If clearer and more well defined zones could be obtained at the higher dilutions of inoculum, a marked increase in sensitivity would be obtained. Clarity of zone may possibly be connected with the growth medium and further work in this connection will be carried out in the near future.

Occasionally a batch of medium is found to give invalid results for no apparent reason. Fortunately, this can be readily seen from the standard curve and in such circumstances the test and medium must be abandoned.

SUMMARY

1. A method for the assay of aneurine in yeast is given based on the plate technique of Bacharach and Cuthbertson.
2. A correct degree of dilution of the organism in the final inoculum is important to obtain a clear and well defined zone of growth.
3. Takadiastase appears to release all the aneurine from yeast and yeast products.
4. Steaming yeast in presence of 0·1 N sulphuric acid gives variable results and papain appears to have a destructive effect on even pure aneurine.
5. The method as advocated is not particularly sensitive, but has been shown to give valid and accurate results, and its precision is at least as high as that of any other method for the determination of aneurine in yeast.

The authors wish to thank Dr. R. H. Marriott and Dr. J. Farquharson for their interest in this work, and the Directors of Beecham Research Laboratories Limited for permission to publish.

They also wish to thank Dr. E. C. Wood and Mr. S. A. Price for helpful criticism.

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DISCUSSION

The Chairman, Dr. A. J. AMOS, congratulated the authors on what appeared to be a very useful assay method and referred to the difficulties hitherto encountered in determining the aneurine content of yeast. He would like to see comparisons between the results obtained by this and by other methods on the same samples.

Mr. J. S. HARRISON quoted some of his results on dried yeast (*Analyst*, 1948, **73**, 539) by the *Neurospora crassa* (9 assays), thiochrome (6 assays) and yeast fermentation methods. The mean estimates, and their 95 per cent. limits of error, were respectively 15·7 ± 4·9, 14·6 ± 1·7 and 14·7 ± 7·0 µg./g.

Dr. E. C. WOOD stated that in view of the difficulty, referred to by Dr. Morris, of checking the absolute accuracy of the cup-plate method on yeast, samples should be assayed both by this method and using rats. Dr. Morris's method of stating the precision of the technique, as the standard error of the results of successive assays on the same sample, was not so informative as the standard error of a single assay calculated from the internal evidence, *i.e.*, the difference between responses to the same dose. Microbiological assays of aneurine employing *Lactobacillus fermentum* in the usual "test tube" technique nearly always showed a certain number of quite anomalous responses in every dose group; had the authors encountered this phenomenon in the cup-plate technique?

Miss A. JONES said that they had not encountered such anomalies in their method.

Mr. S. A. PRICE asked whether the authors had investigated the effect of the pyrimidine and thiazole moieties of the aneurine molecule under their conditions. According to Sarett and Cheldelin these substances were inactive in the "test tube assay" with *L. fermentum* provided that the incubation time was no more than 18 hours. Was this also true of the cup-plate method?

Mr. BACHARACH asked if there was any evidence that these compounds were inactive for mammals. Did their presence, in fact, vitiate an assay?

Dr. E. R. DAWSON also emphasised the importance of ascertaining whether the organism responded to the pyrimidine and thiazole moieties under the conditions of the cup-plate test. In reply to Mr. Bacharach he pointed out that the Labelling of Foods Order specified "Aneurine hydrochloride," not "vitamin B_1."

Dr. W. F. J. CUTHBERTSON enquired whether the enzymic destruction of aneurine had been attempted in the preparation of basal media. This should show advantages in that aneurine may be removed specifically and the enzyme itself readily inactivated without altering any other constituents of the medium. He suggested also that *Streptococcus salivarius* might be tried as a test organism; it should have advantages over *L. fermentum* in being more sensitive to aneurine and less sensitive to the pyrimidine and thiazole breakdown products.

Dr. MORRIS replied that they had not so far investigated the influence on the assay of the pyrimidine and thiazole moieties,* neither had they attempted enzymic destruction of aneurine in the preparation of basal media.

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* But see last four lines of p. 337. Ed.

The Microbiological Assay of Aneurine: An Improved Method Employing *Lactobacillus Fermenti* 36*

By E. E. FITZGERALD and E. B. HUGHES

The method is based on that described by Sarett and Cheldelin,[1] modified subsequently by Barton-Wright[2] and later by Cheldelin, Bennett and Kornberg.[3] The present improvement depends on the destruction of aneurine in an extract of the sample by a modification of the sulphite treatment of Schultz, Atkin and Frey,[4] and the use of this sulphite-treated extract to supplement the standard tubes. Thus all tubes used in an assay contain the same amount of the sample and a comparison is made between untreated extract and sulphite-treated extract plus standard aneurine. In this way inhibitory or stimulating effects due to substances other than aneurine in a sample are counteracted.

Method

The organism used is *Lactobacillus fermenti* 36; stab cultures are maintained by weekly sub-culture on a yeast-water glucose agar containing 10 μg. of aneurine per tube. The cultures are incubated for 30 to 48 hours at 37° C. and stored in a refrigerator (about 5° C.) when not in use.

The basal assay medium (Table I) is that described by Sarett and Cheldelin[1] with addition of 0·5 per cent. of sodium chloride and with a modified Salt Solution B as recommended by Barton-Wright.[2] The preparation and storage of stock solutions from which the medium is prepared are essentially those given in the Analytical Methods Committee's "Report on the Microbiological Assay of Riboflavine and Nicotinic Acid."[5]

The divergence from other methods is in the use of sulphite-treated sample extracts and in the design of the assay.

Table I

Basal medium

(sufficient for 100 tubes)

Photolysed peptone	200 ml.
Casein hydrolysate	2·5 g.
Glucose	20 „
Sodium acetate (cryst.)	10 „
Cystine	0·1 „
Adenine hydrochloride	0·01 „
Guanine „	0·01 „
Uracil	0·01 „
Sodium chloride	5 „
Salt solution A	5 ml.
„ „ B	5 „
Riboflavine	100 μg.
Ca D-pantothenate	100 „
Nicotinic acid	100 „
p-Aminobenzoic acid	100 „
Pyridoxine	100 „
Biotin	0·4 „
Folic acid	0·25 „
Glass-distilled water to	500 ml.
pH	6·5

Photolysed peptone—Dissolve 40 g. of Difco Bacto-peptone in 250 ml. of glass-distilled water and add 20 g. of sodium hydroxide dissolved in 250 ml. of water. Allow the mixture to stand for 24 hours in the light at room temperature, exposing it to strong light (*e.g.*, a 100-watt tungsten filament lamp at 18 inches) for at least 12 hours of this period. At the end of this time add 28 ml. of glacial acetic acid and 11·6 g. of sodium acetate (cryst.). Make up the volume to 800 ml. and preserve the solution under sulphur-free toluene in a refrigerator. This solution will normally keep for a fortnight, but should be discarded if a precipitate forms before this time.

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* Also known as *Lactobacillus fermentum* 36. Ed.

Salt Solution A—Dissolve 25 g. of KH_2PO_4 and 25 g. of K_2HPO_4 in 250 ml. of water. The solution keeps indefinitely in the presence of toluene.

Salt Solution B—Dissolve 10 g. of $MgSO_4.7H_2O$, 0·5 g. of $MnSO_4.4H_2O$ and 0·1 g. of $FeCl_3$ (anhydrous) in 250 ml. of water and add 5 drops of concentrated hydrochloric acid. This solution keeps indefinitely.

PREPARATION OF EXTRACTS—

The sample extract should contain approximately 0·015 µg. of aneurine per ml. The weight of sample taken and the volume to which it is adjusted should be chosen to ensure this. With an unknown sample it may be necessary to make a preliminary test to obtain an approximate value for its aneurine content.

Heat a suitable weighed quantity of the finely divided sample (usually about 1 g.) with 25 ml. of 0·1 *N* sulphuric acid for 30 minutes at 100° C. in a steamer or an autoclave. Cool and adjust the pH to 4·5 with 2·5 *M* sodium acetate solution, using bromocresol green as external indicator. Add 20 mg. each of papain and takadiastase and incubate for 18 to 24 hours at 37° C. under a thin layer of toluene. Heat for 30 minutes at 100° C., cool, make up the volume to 100 ml. and filter through a Whatman No. 1 paper. Take two equal aliquots of the filtrate. Adjust the pH of the first to 6·5, using bromothymol blue as external indicator, and make up with water to such a volume as will give an estimated aneurine content of 0·015 µg. per ml. This is Sample Extract I. To the second aliquot add 4 ml. of a freshly prepared 10 per cent. solution of sodium sulphite crystals, adjust the pH to 5·4 with *N* sulphuric acid, using B.D.H. "4460" indicator externally, make up the volume to about 50 ml. with water and autoclave for 15 minutes at 15 lb. pressure. Cool, and add the exact amount of hydrogen peroxide (A.R. 10 vol.) required to oxidise the excess of sodium sulphite, using as external indicator a freshly prepared mixture of equal volumes of 5 per cent. potassium iodide solution, 1 per cent. starch solution and 50 per cent. sulphuric acid. Adjust the pH to 6·5 (indicator bromothymol blue) and dilute to the same concentration as Sample Extract I. This sulphite-treated extract will be referred to below as Sample Extract II.

PREPARATION OF INOCULUM—

Add 10 µg. of aneurine to 5 ml. of basal medium, dilute with distilled water to 10 ml. and sterilise by autoclaving for 15 minutes at 15 lb. pressure. Make a transfer from a fresh stock culture of the organism to a tube of this medium with a sterile platinum wire. Incubate for 16 to 18 hours at 37° C. and centrifuge aseptically. Pour off the supernatant liquid, suspend the bacterial cells in 10 ml. of sterile saline and dilute 1 drop of suspension in 10 to 25 ml. of sterile saline.

ASSAY PROCEDURE—

Into each of fifteen Pyrex test tubes (18 or 19 by 150 mm.) pipette 5-ml. aliquots of basal medium. To each of five tubes add 2 ml. of Sample Extract I and to each of the remaining ten add 2 ml. of Sample Extract II. One of these ten tubes serves as a blank. Add standard aneurine solution to the other nine at the levels 0·02, 0·03 and 0·04 µg. per tube, using three tubes at each level. Adjust the total volume in each of the fifteen tubes to 10 ml. with distilled water. Plug the tubes with cotton wool and sterilise by autoclaving for 10 minutes at 10 lb. pressure. After cooling, inoculate all tubes except the blank with 1 drop each of the diluted inoculum and incubate them all together in a constant-temperature water-bath at 37° C. for a period not exceeding 18 hours and preferably not less than 17 hours. After incubation remove the tubes, all together, cool for at least 15 minutes in a refrigerator at about 5° C. to arrest growth, and measure the turbidity in the tubes photo-electrically. We have found the Hilger Spekker absorptiometer, with neutral grey filters (H508), satisfactory for this purpose. With this instrument the difference between the optical density of each tube and the uninoculated blank is measured and the scale reading gives a measure of the turbidity produced by bacterial growth.

INTERPRETATION OF RESULTS—

Over the range 0·02 to 0·04 µg. the response of *L. fermenti* to aneurine is nearly linear. The aneurine content of the sample can therefore be deduced by interpolation from the turbidity readings. For example, the protocols of a typical assay were:—

Sample of malt extract—0·996 g. was digested and made up to 100 ml.; 40-ml. aliquots were taken and finally diluted to 100 ml. 2 ml. of Sample Extracts I and II therefore contained 7·97 mg. of the sample.

	µg. Aneurine added	Spekker readings	Mean
Sample Extract I	nil	0·712; 0·713; 0·718; 0·710; 0·725	0·716
Sample Extract II	0·02	0·607; 0·602; 0·585	0·598
	0·03	0·698; 0·710; 0·683	0·697
	0·04	0·807; 0·783; 0·800	0·797

7·97 mg. of sample contain $>$0·03 µg. and $<$0·04 µg. of aneurine.
By interpolation 7·97 mg. of sample contain 0·0319 µg. of aneurine.
Hence the aneurine content of the sample is 4·00 µg. per g.

DISCUSSION

The method of Sarett and Cheldelin was found unsatisfactory for some samples; drifts in the values obtained at different sample levels were obtained because of the stimulatory or inhibitory effect on *L. fermenti* of substances other than aneurine in the sample extracts. The present method was developed with two objects in view. First, to supplement the "standard" tubes with an extract similar to the sample extract except that its aneurine had been inactivated. Secondly, to simplify the design of the assay, for having overcome the stimulatory or inhibitory effects of substances other than aneurine it is no longer necessary to test the sample at more than one level.

Using pure aneurine solutions it was found that the sulphite treatment of Schultze *et al.* destroyed about 95 per cent. of the activity as determined by Sarett and Cheldelin's method but, when the solutions were autoclaved at 15 lb. pressure instead of being steamed for 30 minutes, 99 per cent. of the activity was destroyed in 15 minutes. When this modification of the sulphite treatment was applied to sample extracts supplemented with aneurine no residual activity was found.

If the sulphite treatment has a destructive effect on the stimulatory or inhibitory substances, assays carried out at different levels will show a drift in the results in the same way as in the Sarett and Cheldelin method. Recovery tests were carried out on some samples that had given drifts when assayed by Sarett and Cheldelin's method. In these tests aneurine solution was added in known amount to the weighed sample before extraction and the "fortified" sample was submitted to the full procedure as described above. The aneurine found was from 99 to 107 per cent. of the calculated amount, indicating that no drifts had occurred (Table II).

TABLE II

Sample	Weight of sample per tube mg.	Aneurine content Calculated µg./g.	Aneurine content Found µg./g.	Aneurine recovery %
Wheat germ	1·25	—	21·2	
+ aneurine 23 µg./g.	0·6	44·2	46·8	106
Dried yeast ..	0·8	—	35·3	—
" " + aneurine 35 µg./g.	0·4	70·3	70·3	100
Flour	8·0	—	3·77	—
" + aneurine 2 µg./g.	5·0	5·77	5·70	99
" + " 4	4·0	7·77	8·33	107
" + " 8	2·5	11·77	12·42	106
" + " 16	1·5	19·77	20·89	106

The sulphite treatment introduces into the treated extract (Sample Extract II) a small amount of sodium sulphate, amounting to about 5 to 8 mg. per tube. Tests showed that at some levels sodium sulphate has a slight stimulatory effect on the organism in the presence of aneurine but, by supplementing Sample Extract I with sodium sulphate prepared from sodium sulphite and hydrogen peroxide, the effect was shown to be negligible (Table III).

TABLE III

	Extract I	Extract I supplemented with sodium sulphate
Sample A	2·56 µg. per g.	2·56 µg. per g.
Sample B	2·89 "	3·00 "

Sarett and Cheldelin state that steaming at 100° C. for 15 minutes "is sufficient to keep the test free from contamination for the short time that it is run. Steaming, in contrast to autoclaving at higher temperature, does not darken the medium and decreases the possibility of any thiamine destruction." When assay tubes were autoclaved for 10 minutes at 10 lb. pressure no destruction of aneurine occurred and there was only slight darkening of the medium. This treatment has been found to be satisfactory in all other microbiological assays carried out by us and was adopted throughout the present work.

L. fermenti shows very little growth in the first 20 to 24 hours of incubation in the basal medium to which the hydrolysis products of aneurine have been added (sulphite-treated yeast extract was actually used). After this period the organism grows rapidly, reaching full growth in a further 40 hours (Fig. 1). The growth in the first 20 hours, although small, was difficult to account for until it was found to be due to residual aneurine in the alkali-treated peptone in the medium. When the peptone was prepared by treatment with sulphite a very low "blank" was obtained in the first 20 hours of incubation. Under normal assay conditions, that is, in presence of about 0·03 μg. of aneurine per tube, the lag phase is about 6 hours, the logarithmic phase is passed in a further 10 hours, and after this growth continues more slowly. Thus, with an incubation time of 18 hours, growth is independent of aneurine degradation products, whilst the effect of intact aneurine is approaching its maximum.

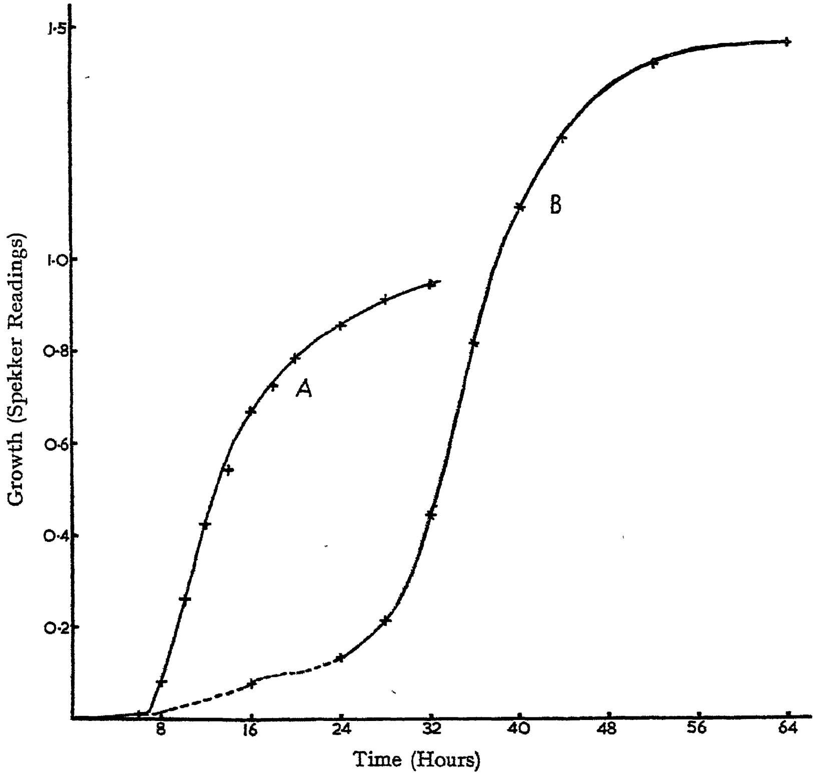

Fig. 1. A. With 0.03 μg. of aneurine per tube. B. In sulphite-treated yeast extract (no added aneurine)

TABLE IV

Sample	Separate assay results (μg. aneurine per g.)	Mean
Flour	3·45, 4·18, 3·73, 3·73, 3·62	3·74
Wheat germ	23·3, 23·7, 21·2	22·7
Dried yeast	37·6, 33·8, 37·5, 35·3	36·1
Malt extract	4·09, 4·01	4·05

This work indicated that by treating natural products such as peptone and yeast extract with sulphite it should be possible to devise a simpler basal medium with a shorter list of synthetic supplements; this is now being investigated.

The method described in this paper has been applied to a wide range of food materials. The results have been satisfactorily reproducible (Table IV) and have agreed reasonably well with parallel chemical assays (Table V).

TABLE V

Sample	Microbiological method. Aneurine μg. per g.	Thiochrome method.* Aneurine μg. per g.
Flour	3·47	2·75
Wheat germ	22·7	18·9
Wheat germ preparation 1	24·0	20·5
„ „ „ 2	17·7	14·1
„ „ „ 3	19·7	15·8
Barley kernels	1·92	1·33
Rolled oats	6·4	6·1
Malt extract	4·05	3·37
Yeast extract	57·6	55·8
Dried yeast	36·1	29·8
Full cream milk powder	3·49	3·55
Skim milk powder	4·83	3·40
Dried egg	2·41	2·9
Lean pork	12·1	12·6
Ox kidney	4·2	4·4
Ox liver	2·9	3·1
Canned roast pork	1·46	1·46
Vitamin tablet	537	339

* Using Decalso for purification of the extracts.

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A Scheme for the Chromatographic Examination of Propellant Explosives

BY T. C. J. OVENSTON

THE application of the chromatographic method to the examination of some complex propellant compositions from foreign sources has demonstrated very clearly the value of this elegant technique in aiding the solution of the analytical problems involved. Used in conjunction with appropriate streak tests applied to the extruded column, the value of the method from a qualitative point of view lies in the fact that not only are colour tests employed on the separated ingredients, but the characteristic adsorption affinity of each ingredient is noted. The selectivity is thus greatly increased in comparison with the use of colour tests alone, particularly when the latter are applied to the mixture of ingredients to be found in the ether extract of the propellant.

The present paper describes a scheme for the chromatographic detection of any of twenty-four possible ether-soluble propellant ingredients in the presence of one another, and discusses briefly a few quantitative applications.

PROPELLANT INGREDIENTS STUDIED

The twenty-four ether-soluble propellant ingredients included in the present scheme are listed in Table I, together with the appropriate abbreviations. The list is arranged in alphabetical order of abbreviation for easy reference as these abbreviations are used in other parts of this paper.

TABLE I

PROPELLANT INGREDIENTS AND THEIR ABBREVIATIONS

Chemical name	Abbreviation
N : N-Diphenylurea (Acardite)	AC
N : N′-Diphenylurea (Carbanilide)	CBAN
Carbazole	CBZ
Diamyl phthalate	DAP
Dibutyl phthalate	DBP
Diethylene glycol dinitrate	DEGN
Diethyl phthalate	DEP
Dimethyl phthalate	DMP
2 : 4-Dinitrotoluene	DNT
Diphenylamine	DPA
Diphenylformamide	DPF
N-Nitrosodiphenylamine (Diphenyl-nitrosoamine)	DPN
N : N-Diphenylurethane	DPU
N : N′-Diphenyl-N : N′-diethylurea (Carbamite or Ethyl Centralite)	EC
N : N′-Diphenyl-N : N′-dimethylurea (Methyl Centralite)	MC
Mineral jelly	MJ
1-Nitronaphthalene	MNN
Nitroglycerine	NG
2-Naphthyl-methyl-ether	NME
N-Phenyl-N-benzylurethane	PBU
N-Nitroso-N-ethylaniline (Phenyl-ethyl-nitrosoamine)	PEN
N-Phenyl-N-ethylurethane	PEU
2 : 4 : 6-Trinitrotoluene	TNT
N-(2-Tolyl)-urethane	TU

GENERAL EXPERIMENTAL TECHNIQUE

The comparative chromatographic characteristics of these compounds were investigated, and tables showing orders of adsorption with various binary developing solvents were constructed.[1] This information formed the basis of the present scheme of analysis. The following special points regarding materials and technique are noted.

APPARATUS—The chromatographic tubes are most conveniently made from ordinary uniform glass tubing, in lengths of 25 cm. No stricture is required, so that extrusion of the column is not impeded. The tube is held in an adaptor by a rubber ring, and rests on a filter plate in the adaptor. A receiving flask may be attached to the bottom of the adaptor (a ground glass joint is very convenient here), and a side-arm on the adaptor allows suction to be applied to the base of the column.

ADSORBENT—Except for the detection of mineral jelly, the same adsorbent is used throughout the scheme. This consists of a mixture of equal parts of silica gel and Celite 535, uniformly activated by heating at 120° C. The silica gel is a particularly fine grade, marketed by Messrs. Silica Gel, Ltd. The filter aid, Celite 535, is marketed by Messrs. Johns-Manville Co., Ltd. A closely similar adsorbent has been described by LeRosen.[2] The success of the present scheme of analysis depends, of course, on the reproducibility of this adsorbent, but experience suggests that there should be no difficulty in this matter.

SIZE OF COLUMN—The scheme is designed for use with columns 1 cm. wide by 15 cm. long. The actual length is not very critical (owing to the employment of "indicator zones"), but any appreciable difference in width from that stated above should be allowed for by suitable adjustment of the volumes of developing solvents prescribed. Thus, for a width of 1·1 cm., a prescribed volume of 100 ml. of developing solvent becomes 121 ml.

SOLVENTS—The developing solvents employed consist mainly of binary mixtures of light petroleum, benzene and ethyl ether. The light petroleum recommended is that fraction boiling between 40° and 50° C. Other than redistillation, special purification is not necessary here. The benzene should be of the pure "crystallisable" grade, and the ethyl ether should be dry and recently distilled over caustic alkali.

PACKING OF COLUMN—A plug of cotton wool is placed at the bottom of the glass tube, and sufficient adsorbent is poured in to give a column 2 to 3 cm. high. Suction is gently applied and then released again, and the tube is tapped to level the surface of the powder. Suction is then applied again and a further similar quantity of adsorbent is added in a thin stream. The suction is released again and the tube tapped as before; then suction is reapplied and the tube again tapped to assist settlement. This procedure is repeated until the desired height is obtained.

STREAK TESTS EMPLOYED

Colour reactions are given by most propellant ingredients, and some of these are readily adaptable as chromatographic "streak" tests (the *Pinselmethode* of Zechmeister *et al.*[3]). Excluding mineral jelly, for which such a test is unnecessary, streak tests have been devised for all the compounds under consideration.

A method of application favoured by the author is to fill a small pipette (of about 4 inches over-all length) with the reagent and, starting at one end of the extruded column, draw the tip slowly along the column, allowing the reagent to flow freely through the tip, the size of which is chosen to suit the viscosity of the reagent. In this way narrow and uniform streaks can easily be made. By moving the column round, several different streaks may be made on the same column.

The *streak reagents* used in the present scheme are as follows:—

*S.R.*1. 0·5 per cent. solution of potassium dichromate in 60 per cent. w/w sulphuric acid.

*S.R.*2. 0·5 per cent. solution of potassium dichromate in water, followed by a superimposed streak of 90 per cent. w/w sulphuric acid.

*S.R.*3. 1 per cent. solution of diphenylamine in concentrated sulphuric acid.

*S.R.*4. 6 *N* aqueous sodium hydroxide.

*S.R.*5. bromocresol green solution (B.D.H.).

The first four of these reagents are more or less specific for certain groups of compounds. Descriptions of the streaks obtained with the compounds under consideration are given in Table II. The phthalic esters are not affected by any of these four reagents, and their detection by this method has proved to be difficult. Of many reagents tried, bromocresol

TABLE II

STREAK TESTS OF THE PROPELLANT INGREDIENTS

(excluding phthalic esters and mineral jelly)

Propellant ingredient	Streak reagent S.R.1	S.R.2	S.R.3	S.R.4
EC	Red (*a*)	Red (*a*)	No colour	No colour
MC	Red (*a*)	Red (*a*)	"	"
AC	Blue (*a*)	Blue (*a*)	"	"
CBAN	Olive-green	Olive-green	"	"
CBZ	Green (*a*)	Green (*a*)	"	"
DPA	Blue (*a*)	Blue (*a*)	"	"
DPU	No colour	Blue, fading (*a*)	"	"
PEU	Pink, fading	Magenta, fading (*a*)	"	"
PBU	No colour	Magenta, fading	"	"
TU	Pink, fading	Magenta, fading (*a*)	"	"
NME	Purple-brown (*a*)	Purple-brown	"	"
DPN	Blue (*a*) (*b*)	Blue (*a*) (*b*)	Blue (*b*) (*c*)	"
PEN	Golden yellow (*a*) (*d*)	Golden yellow (*d*)	Faint blue (*c*)	"
DPF	Faint blue-grey (*e*)	Faint blue-grey	No colour	
DNT	No colour	No colour	"	Pale violet, fading to pale brown (*f*)
TNT	"	"	"	Brick red (*a*)
MNN	"	"	Orange-red (*a*) (*b*)	No colour
NG	"	"	Dark blue (*a*) (*g*)	"
DEGN	"	"	Dark blue (*g*)	"

NOTES—(*a*) These tests are particularly sensitive.

(*b*) Given also by concentrated sulphuric acid alone.

(*c*) The test for aromatic N-nitrosoamines can be made very sensitive by diluting 2 parts of the reagent with 3 parts of water and using while still warm. (The DPA crystallises out on cooling.) Under these conditions nitric esters give no streak.

(*d*) Colour develops after a few seconds.

(*e*) This test is rendered very sensitive by first streaking with S.R.4 and then superimposing the streak of the dichromate reagent.

(*f*) This test is rendered more sensitive by saturating the streak reagent with acetone before application.

(*g*) The test can be made more sensitive, if necessary, by first streaking with S.R.4 and then superimposing the streak of S.R.3.

green solution has proved the most satisfactory. It can be applied to a silica gel - Celite column either immediately on extrusion (as normally), or 10 to 15 minutes after extrusion, when the excess of solvent has evaporated. The test behaves differently in the two cases, and it is advantageous to perform it in both ways. In each case a sky-blue streak is produced on the column by the indicator itself. This background colour remains throughout the test, and phthalic ester zones are indicated by a fading or a change in tint. This test will detect quantities of phthalic esters down to 1 mg. on silica gel - Celite columns of 1-cm. diameter. The reagent is by no means specific, and many other compounds listed in Table I will give some change of background with this indicator. Used in parallel with other streak tests, however, it has a definite value. Details of the test as applied to phthalic esters are given in Table III.

TABLE III

STREAK TESTS OF THE PHTHALIC ESTERS

Phthalic ester	S.R.5 applied immediately on extrusion	S.R.5 applied 10 minutes after extrusion
DAP	Sky blue background fades in a few minutes, particularly at leading and trailing edges of zone, latter edge showing widest fading. Optimum time for observation is 20 minutes after application, when main portion of zone is indicated by a greenish tint bordered by completely faded edges	Sky blue background darkens and assumes a greenish tint, at first without fading. Optimum time for observation is 10 minutes after application, when edges of main greenish zone are sharply defined by completely faded areas. These edges locate the actual borders of the DAP zone more accurately than the corresponding edges produced when S.R.5 is applied immediately on extrusion
DBP	As for DAP	As for DAP
DEP	As for DAP except that greenish tint is very faded	As for DAP but somewhat less sensitive
DMP	As for DEP	As for DEP except that original darkening is negligible, and final faded zone shows no obvious greenish tint

SCHEME FOR THE CHROMATOGRAPHIC DETECTION OF INGREDIENTS IN THE ETHER EXTRACT OF A PROPELLANT

This scheme has been developed in view of the possibility of any number of the compounds under consideration being present together. The tests to be described are made on suitable portions of the ether extract of the propellant after removal of the ether *in vacuo*. Each portion is taken up in the smallest practicable volume of the appropriate developing solvent and transferred to the column for chromatography.

Four simple chromatograms suffice to reveal the presence of any of the ingredients named in Table II, except in the presence of nitric esters, when a slight modification is necessary. For the more difficult separation of the phthalic esters a three-column procedure is employed. The approximate location of the various zones is shown in Fig. 1. Although these zones may be displaced somewhat by such factors as slight variations in activity of the adsorbent, interference with the developing rate due to interaction between certain solutes when present in excess, and so on, their relative positions will remain substantially as indicated.

A. PROCEDURE FOR INGREDIENTS OTHER THAN PHTHALIC ESTERS AND MINERAL JELLY—

Column 1—for 2-naphthyl-methyl-ether, 1-nitronaphthalene, diphenylamine, carbazole and nitroglycerine.

(i) Develop with 25 ml. of a mixture of 20 per cent. of benzene in light petroleum and post-wash with 20 ml. of pure light petroleum before extruding.

(ii) A faint yellow zone just more than half-way down the column indicates the possible presence of 1-nitronaphthalene.

(iii) S.R.1 detects 2-naphthyl-methyl-ether, diphenylamine and carbazole.

(iv) S.R.3 detects nitroglycerine and confirms the presence of 1-nitronaphthalene.

Column 2—for 2 : 4-dinitrotoluene, 2 : 4 : 6 trinitrotoluene, N-nitrosodiphenylamine, N-nitroso-N-ethylaniline and diethylene glycol dinitrate.

(i) Develop with 80 ml. of a mixture of 35 per cent. of benzene in light petroleum and post-wash with 20 ml. of pure light petroleum before extruding.

(ii) S.R.3 detects diethylene glycol dinitrate (near top of column). A blue streak at the bottom of the column indicates the trailing edge of the nitroglycerine zone. (This zone is normally entirely or almost entirely washed through.)

(iii) The presence of nitroglycerine or diethylene glycol dinitrate will affect the development of the other zones on this column. 2 : 4-Dinitrotoluene, for instance, if present, may remain undetected owing to displacement and spreading of the zone by the increased development produced locally on the column by nitroglycerine. Therefore, if nitric esters have been detected (on either Column 1 or Column 2), they should be removed as described in (iv) below. In absence of nitric esters the column prepared in (i) may be used for the detection of the remaining compounds, as described in (v) and (vi) below.

(iv) Place on a new column a fresh portion of propellant extract dissolved in 1 ml. of a mixture of 10 per cent. of ethyl ether in light petroleum, containing 0·2 mg. of 2 : 4 : 6-trinitro-N-ethylaniline as an indicator. Develop with further quantities of this solvent mixture until the leading edge of the yellow zone of the indicator is within 0·5 cm. of the bottom of the column. (About 50 ml. of solvent are required.) The nitric esters remain adsorbed on the column, which should be discarded. Add 0·5 ml. of B.P. paraffin to the eluate and evaporate *in vacuo* in dim light (the nitrosoamines are somewhat photo-sensitive). Re-chromatograph as described in (i) above, and then proceed as described in (v) and (vi).

(v) Streak with S.R.4 saturated with acetone to detect the di and trinitrotoluenes.

(vi) Streak with S.R.1 to detect N-nitrosodiphenylamine and N-nitroso-N-ethylaniline. Confirmation may be obtained by streaking with the modified S.R.3 described in Note (*c*) at foot of Table II.

Column 3—for ethyl Centralite, diphenylformamide, methyl Centralite, carbanilide and Acardite.

(i) Develop with 40 ml. of a mixture of 20 per cent. ethyl ether in light petroleum; then extrude the column.

(ii) Streak with S.R.1 to detect ethyl and methyl Centralite, carbanilide and Acardite. Diphenylformamide may give a faint colour.

(iii) Streak with S.R.4 and superimpose a streak of S.R.1 to detect diphenylformamide (see Note (*e*) at foot of Table II).

Column 4—for urethanes.

(i) Develop with 40 ml. of a mixture of 0·25 per cent. of ethyl ether in benzene and post-wash with 20 ml. of light petroleum before extruding.

(ii) Streak with S.R.2 to detect the urethanes. A faint blue streak near the top of the column may appear if diphenylformamide is present, but this should not be confused with the blue streak given by N : N-diphenylurethane which would normally appear just more than half-way down the column.

(iii) Streak with S.R.1 to confirm N-phenyl-N-ethylurethane and N-(2-tolyl)-urethane.

B. PROCEDURE FOR PHTHALIC ESTERS—

(i) Dissolve a small portion of the propellant extract in 1 ml. of benzene containing 0·5 mg. of picramide and 0·2 mg. of 4-nitroaniline (as indicators), and place on the column. Develop with benzene until the yellow zone of picramide (the lower of the two indicator zones) has just been washed through the column. (About 60 ml. of benzene should be required). At this stage the yellow zone of 4-nitroaniline should be not less than 7 cm. from the bottom of the column. The percolate contains the whole of the nitric esters and any of the more weakly adsorbed ingredients, and should be discarded.

(ii) Using a fresh receiving flask, continue development without delay, this time using a mixture of 2 per cent. of ethyl ether in benzene, until the leading edge of the yellow zone of 4-nitroaniline is within 1 cm. of the bottom of the column. (About 40 ml. of this developing solvent should be required.) The column retains the diphenylformamide and more strongly adsorbed ingredients, and may be discarded.

(iii) The percolate from (ii) above contains any phthalic esters and urethanes present. Evaporate off the solvent, dissolve in 1 ml. of a mixture of 10 per cent. of ethyl ether in light petroleum containing 0·2 mg. of 2 : 4 : 6-trinitro-N-ethylaniline, and place on a fresh column. Develop with a mixture of 10 per cent. of ethyl ether in light petroleum until the leading edge of the yellow zone of the indicator is about 2 cm. from the bottom of the column. (About 50 ml. of the developing solvent should be required.) The percolate contains any diamyl phthalate or dibutyl phthalate completely separated from all the other ingredients under consideration. This should be treated as described in (vi) below.

(iv) Extrude the column from (iii) above and streak with S.R.5 to detect diethyl phthalate and dimethyl phthalate (Column 5, Fig. 1).

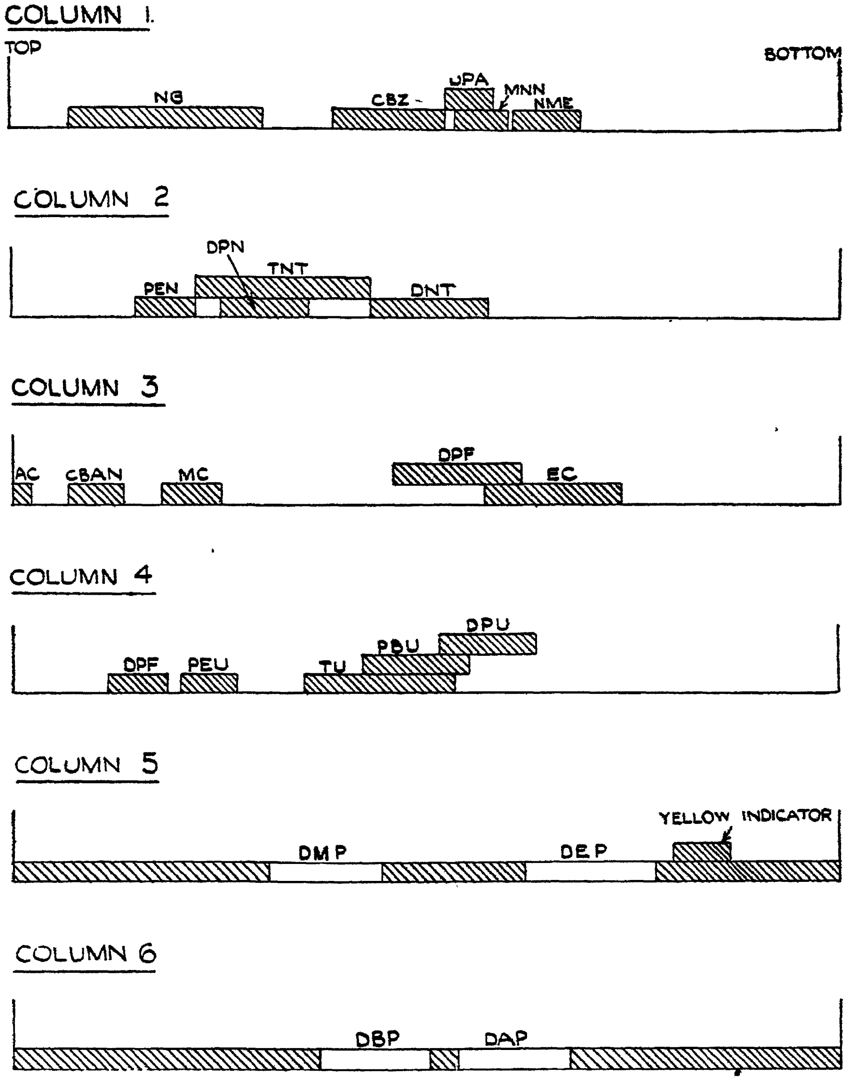

Fig. 1. Guide to the Location of Zones on the Developed Columns

(v) Streak with S.R.2 to detect any urethanes adsorbed near the bottom of the column. This will prevent confusion should a doubtful change in tint of the S.R.5 streak occur in this region, since urethanes may produce such an effect.

(vi) Evaporate the percolate from (iii) above, and take up the residue in 1 ml. of a mixture of 5 per cent. of ethyl ether in light petroleum. Place on a fresh column and develop with 35 ml. of this solvent mixture.

(vii) Extrude and streak with S.R.5 to detect diamyl phthalate and dibutyl phthalate (Column 6, Fig. 1).

C. PROCEDURE FOR MINERAL JELLY—

Mineral jelly can be detected, and at the same time estimated, by the aluminium oxide column method described in an earlier note.[4] No further description need be given here.

CONFIRMATORY TESTS

When the presence of a compound is indicated by a streak test, sufficient may be recovered by elution (after removal of the small portion penetrated by the streak reagent) to allow appropriate confirmatory tests to be made. The large number of existing colour "spot" tests are useful in this respect. When a nitric ester has been detected the refractive index will serve to confirm either nitroglycerine or diethylene glycol dinitrate, or will indicate perhaps that some other nitric ester should be suspected. In cases of doubt, a satisfactory method of checking identity is to mix the substance with a small amount of authentic material and re-chromatograph, when the development of a single zone showing no signs of separation (as indicated by the streak on the extruded column) confirms identity.

Most of the ingredients under consideration are best identified by their ultra-violet absorption spectra, which can readily be obtained on an alcoholic eluate. It is of advantage, therefore, to have available for purposes of reference a chart of absorption spectra of all likely ingredients. In applying the present scheme to the analysis of an unknown propellant the possibility of finding an ingredient not included in Table I should, of course, be borne in mind. The first indication of this may be the appearance of a positive streak at an unusual position on one of the columns. In such circumstances the plotting of an absorption spectrum may be all that is needed for final identification.

With the phthalic esters confirmatory tests are essential. However, the location on the column, coupled with the fluorescein test and measurement of refractive index, should suffice to identify the compound.

QUANTITATIVE APPLICATIONS

Having established the qualitative composition of the propellant extract, a method for the quantitative estimation of the components would normally be devised. If the ingredients are readily estimated by standard methods there seems to be no particular advantage in trying to use chromatographic methods. However, the analysis of the more complex mixtures will be greatly assisted by the use of chromatographic separations. The qualitative scheme already described and the "adsorption series" recorded in a separate paper[1] will suggest the appropriate means of chromatographic development to be employed for a given separation. The following examples will suffice to show the value of this method.

(*a*) Mixtures of nitroglycerine and diethylene glycol dinitrate can be separated quantitatively by development with a mixture of 35 per cent. of benzene in light petroleum.

(*b*) Acardite, methyl Centralite, ethyl Centralite and diphenylamine can be quantitatively resolved on a single column by development with a mixture of 10 to 20 per cent. of ethyl ether in light petroleum. The diphenylamine passes completely into the percolate leaving the other three compounds on the column in three clearly separate zones.

(*c*) N : N-Diphenylurethane is readily separated from the other urethanes considered by using 0·5 per cent. solution of ethyl ether in benzene. The separation of the remainder is difficult, but can be accomplished with the same solvent on a long column.

(*d*) Diphenylamine, 1-nitronaphthalene and naphthyl methyl ether cannot be separated satisfactorily on silica gel. However, quantitative separation of these three compounds is readily obtained (in this order—top to bottom) using a mixture of 20 per cent. of benzene in light petroleum on a column of activated aluminium oxide.

(*e*) The zones of diphenylformamide and ethyl Centralite overlap slightly when developed with ethyl ether - light petroleum mixtures. By using a 1 per cent. solution of ethyl ether in benzene, however, the relative positions of the zones are reversed, and complete separation is easily obtained.

It should be mentioned here that Schroeder[5] has recently published the results of work on the application of chromatography to the quantitative separation of some propellant ingredients. The separated compounds, after elution, were determined spectrophotometrically in alcoholic solution. Only one specific example is provided with a full experimental procedure, this being the separation and determination of diphenylamine and diethylphthalate in a powder containing also nitrocellulose and nitroglycerine, but many other interesting examples are briefly described. It is of interest that Schroeder states that (referring to the specific example just mentioned) "usually, the results from duplicate aliquot portions of the same extract agreed to within 1 per cent. of the amount of the compound, and that the values from duplicate extracts of the same powder had a maximum spread of about 3 per cent. of the compound present," and adds later: "These results are representative of those which we obtained with quantitative chromatographic-spectrophotometric procedures for other compounds." In the present author's experience with the spectrophotometric finish to similar chromatographic separations this statement of the degree of precision obtainable is a fair one.

The work described in this paper was carried out at the Naval Ordnance Inspection Laboratory before its transfer from Holton Heath. The author desires to acknowledge the valuable assistance rendered by Mr. J. R. Smith with the experimental work.

This article is published with the approval of the Lords Commissioners of the Admiralty, but the responsibility for any statements of fact or opinions expressed rests solely with the author.

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A Combustion Method for the Estimation of Carbon Black in Compounded Rubber

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INTRODUCTION

METHODS in general use for the estimation of carbon black in compounded rubber fall into three groups, *viz.*:—

(*a*) Those in which the rubber matrix, together with most of the compounding ingredients, are degraded or dissolved by hot nitric acid.
(*b*) Those that involve the removal of the rubber and some compounding ingredients by means of an inert solvent, usually at an elevated temperature.
(*c*) Combustion methods in which the organic ingredients of the mixing and the rubber itself are destroyed by dry distillation in an inert atmosphere.

In all three procedures the carbon black is left in association with some mineral matter, and its final assay is carried out either by collecting the black and other unattacked residue in a filter crucible and weighing before and after combustion in an oxidising atmosphere, or by combusting the carbon black to carbon dioxide which is collected and weighed in an absorption train of orthodox type.

Methods of the first group are very old-established in rubber analysis, some of the earliest work having been carried out prior to 1914 by H. Williams Jones.[1] The difficulty of retaining finely dispersed black on the filter pad has always been a troublesome feature, however, and as an improvement in this respect, Hammond[2] proposed the use of a filter pad of ignited kieselguhr supported on a layer of asbestos. This modification appears successful in a limited number of cases, but it is debatable whether the use of kieselguhr as a filtering medium provides any benefit over a properly compacted asbestos pad (*cf.* Curran[3]).

The strongly adsorptive nature of many grades of carbon black leads to retention of decomposition products of the rubber, which in turn gives rise to fictitiously high values for the final carbon black figure. The older method of dealing with this positive error was to employ a purely empirical factor of approximately 0·95, a correction which appears to be a mean value between those for the highly adsorptive channel blacks and the low volatile lamp blacks, these blacks being the only types then in common use.

Alternatively, it has been proposed to remove such adsorbed substances by washing the separated carbon black with powerful solvents such as hot acetone or chloroform.[4] The experience of this and other laboratories, however, is that the addition of such washing agents brings about immediate dispersion of the carbon black into the filtrate, necessitating troublesome refiltration with attendant inaccuracies. Even this modification, however, does not always solve the problem of adsorbed substances, for Oldham and Harrison[5] advocate the use of a correction factor of 0·95 *in addition to* washing the carbon black with acetone and chloroform.

In the present B.S.I. method,[6] interference from compounding ingredients that undergo a change in weight on heating (*e.g.*, china clay) is eliminated by oxidising the separated carbon black in a combustion tube to carbon dioxide which is collected and weighed in a soda-lime tube. Since the method estimates only "free" carbon, however, and in no way indicates the type of carbon black, the final adjustment of this figure to that for the carbon black originally present is entirely speculative.

The filtration difficulties encountered in nitric acid decomposition methods have led to proposals to use an inert solvent of high boiling-point for removal of the rubber matrix[7,8]; some improvement is gained thereby, but organic matter is almost always entrained by the insoluble residue, leading to high and variable results. A further disadvantage is that mineral ingredients of the mixing appear substantially in their original state, and their behaviour in subsequent steps of the assay is to a large extent unpredictable. Solvent methods, therefore, on the whole have not received general support.

Combustion methods, compared with the other types mentioned, have the merit of speed, and mainly for this reason have attracted the attention of analysts, particularly for control testing. Namita[9] recommends a simple procedure of heating the compounded rubber for a specified time in a Rose's crucible into which a stream of nitrogen is fed. The loss in weight after re-heating in an oxidising atmosphere, after suitable correction, is assumed to represent carbon black.

The work of Marty[10] gives the first published information on modifications to the above simple technique. He worked with a closed tube in which the sample was contained in a boat, and a current of nitrogen was passed through the tube. The weight of carbon was finally determined by the difference method.

The investigations reported here were carried out with the object of improving still further the basic process proposed by Marty, while retaining as far as possible the advantages of speed, and the use of a small sample. The increasing adoption of rubber-like synthetic materials on the one hand, and the variety of grades of carbon black now available to manufacturers on the other, have sensibly increased the task to the analyst, and have revealed some of the shortcomings of the older methods. It is hoped that this paper will provide a useful line of attack on a problem of rapidly increasing importance and complexity.

EXPERIMENTAL

1. OUTLINE OF METHOD—

A stream of nitrogen from a cylinder is fed through a purifying train into the first combustion tube, where pyrolysis of the volatile organic constituents of the rubber compound takes place. The residue in the combustion boat consists substantially of carbon black and mineral matter. After weighing, the boat is transferred to the second combustion tube, where oxidation to carbon dioxide takes place. The carbon dioxide is trapped in an absorption train of orthodox type and weighed. The boat is again weighed after the oxidation stage and the carbon content obtained by difference. It will be shown later how a consideration of these two values for the "carbon" leads to (*a*) a figure for the total carbon black content of the compound, (*b*) an indication of the type of carbon black used and (*c*) in favourable instances, an estimate of the ratio of blacks in a mixture of two types.

A detailed account of the apparatus and procedure, together with a diagrammatic representation of the assembly is given on p. 359.

2. INERT ATMOSPHERES AND GAS PURIFICATION—

The first requirement of the gas that provides the inert atmosphere in the pyrolysis train is complete freedom from oxygen, since preliminary investigation showed the extreme susceptibility of the carbon to oxidation at the temperature employed. Coal gas and hydrogen, although otherwise suitable, were not considered on account of the serious fire risk.

Carbon dioxide meets the requirements of inertness and non-inflammability, but there is no clear evidence that the reaction $CO_2 + C \rightarrow 2CO$, which takes place readily at 900° C. does not also proceed to some extent at the temperature of the initial pyrolysis (600° C.). Also there is the possibility that the carbon dioxide might combine with certain metal oxides normally used in rubber compounding and seriously affect the weighings.

Compressed nitrogen from a cylinder, after further purification was found satisfactory. Although the "pure" material was used, preliminary experiments with carbon black alone indicated considerable continuous losses due to traces of oxygen in the gas. Further purification was carried out by a two-stage process comprising (*a*) a pair of Drechsel washing bottles containing alkaline pyrogallol and (*b*) a 30-cm. column of reduced copper turnings maintained at approximately 500° C. A small wash-bottle containing concentrated sulphuric acid was interposed between the pyrogallol and the copper.

Some purification of the oxygen used in the second stage is also necessary; this was accomplished by a pair of U-tubes containing soda-asbestos and Anhydrone.

3. COMBUSTION TEMPERATURES—

For the initial dry distillation stage a temperature must be chosen that will ensure the removal of the organic constituents of the compound without bringing about their breakdown into carbon, which would, of course, produce high values for the "carbon" content of the rubber compound. Too low a temperature will entail an unduly long distillation time, and analytical errors may result from the failure to remove certain acid-forming substances such as sulphur. Nitrogen-containing compounds such as acrylo-nitrile polymers may give rise to oxides of nitrogen during the next (oxidation) stage, if not first removed by distillation; this also would lead to high results for the carbon content, unless the oxides of nitrogen are removed by a suitable reagent.

A temperature of 600° C. was found most suitable and it was concluded after investigation that a variation up to +30° was permissible. A total heating time of 10 minutes was found adequate to remove volatile substances at this temperature.

In the oxidation tube a temperature of 900° C. for 15 minutes was found suitable. Below 900° C. complete oxidation of the carbon becomes uncertain under the experimental conditions employed; higher temperatures tend to cause rapid deterioration of the furnace windings and to give rise to difficulties with fusion of the copper oxide tube filling.

The amount of inherent volatile matter of the carbon black that is evolved over the range 600° to 900° C. is used to indicate the identity of the type of carbon black used in the compound, and from this and the fixed carbon content as determined from the weight of carbon dioxide absorbed, the proportion of carbon black originally present in the compound may be calculated (see later).

4. ABSORPTION SYSTEMS—

(*a*) *Nitrogen tube*—The purpose of the absorption train at the exit here is merely to prevent the escape of unpleasant decomposition vapours from the rubber and compounding ingredients into the atmosphere of the laboratory. For this purpose Marty has suggested xylene as absorbent[10]; it was found necessary, however, with the high gas flow rates used, to draw off escaping vapours not trapped by the xylene by means of a filter pump.

(*b*) *Oxygen tube*—The main purpose of this absorption train is, of course, to absorb quantitatively the carbon dioxide formed by the combustion of the carbon black. Soda-asbestos ("Carbosorb" brand) was the absorbing agent used, contained in a stoppered U-tube. To prevent interference from oxides of nitrogen the U-tube is preceded by a small Arnold bubbler containing potassium permanganate solution and sulphuric acid, with an intermediate U-tube containing magnesium perchlorate for drying purposes. The absorption train is concluded by another Arnold bubbler containing concentrated sulphuric acid.

5. CALCULATION OF RESULTS—

(*a*) *Carbon residues from rubber and organic compounding ingredients*—For quantitative results to be obtained by the method outlined it is of obvious importance that organic constituents of the compound should leave either no residue at all apart from mineral matter after heating in nitrogen at 600° C., or should leave a constant proportion of carbonaceous residue whose influence on the final carbon figure can be calculated.

From none of the rubbers and rubber-like materials investigated were the non-mineral constituents completely volatilised. A list of the polymers tested, and the proportion of residual carbon formed, is given in Table I.

TABLE I

Type of polymer	C, %*	
Natural rubber, plantation crepe	0·27,	0·27
Natural rubber, smoked sheet	0·24,	0·22
Butadiene - styrene copolymer (GR–S)	0·23,	0·22
*iso*Butene - isoprene copolymer (GR–I)	0·10,	0·10
Butadiene - acrylonitrile copolymer (Standard Perbunan)	1·5,	1·5
Butadiene - acrylonitrile copolymer (Perbunan 35)	1·9,	1·9
Butadiene - acrylonitrile copolymer (Hycar O.R.15)	3·6,	3·7
Thioplast (Thiokol F.A.)	2·6,	3·1
Polyvinyl acetate	2·7,	2·6
Polyvinyl chloride	5·7,	6·1
Chloro-butadiene (Neoprene)	15·4,	12·3

* This represents the proportion of residual carbon after heating the polymer in nitrogen at 600° C. for 10 minutes.

For most of the materials the amount of residual carbon produced is small and reasonably constant in amount, and for natural rubber, GR–S and GR–I, little error would be introduced by omitting this correction from the subsequent calculation.

The butadiene - acrylonitrile series show distinctly higher values and also some variation between different grades. It becomes imperative, therefore, when determining carbon black in compounds based on these materials to make a correction for the amount and type of rubber present. Experience with these compounds has shown that for this reason it is unwise to ascribe a higher degree of accuracy than ±2 per cent. of the carbon black content; with low loadings, for example about 5 per cent. of black, the experimental error may be even higher.

As would be expected, the chlorine-containing polymers offer considerable resistance to dry distillation, and with them the residual carbon is undesirably high and inconsistent in amount. It would seem, therefore, that the method is inapplicable to these polymers and further investigations with them were not pursued.

Most compounding ingredients of an organic nature are extractable with acetone or chloroform, and hence by determining the carbon black content on the extracted material, interference from these sources may be avoided. Factice (sulphur-vulcanised oil), however, will resist extraction with these solvents, but dry distillation of this material has shown it to be almost completely volatile, the residual carbon after combustion in nitrogen being constant at about 0·5 per cent. As factice is used in rubber compounding to only a minor extent, interference from this source will usually be negligible.

(*b*) *Influence of inorganic fillers*—The majority of inorganic compounding ingredients likely to be encountered in rubber compounded with carbon black will not change in weight between 600° and 900° C. Thus magnesium carbonate readily loses carbon dioxide below 600° C. and its presence therefore will not influence subsequent weighings.

China clay loses 10 per cent. of its weight at 600° C. and a further 2 per cent. at 900° C. In the method described, the loss in weight on heating in oxygen, therefore, will be increased by an amount corresponding to 2 per cent. of the clay present, and in making the final computation of the original carbon black content a correction must be applied based on the china clay content of the compound.

Calcium carbonate is not appreciably decomposed below 600° C., but loses all its combined carbon dioxide between 600° and 900° C. The carbon dioxide thus liberated will be absorbed in the soda-asbestos tube and must be corrected for in the final result by computing its value from the amount of calcium carbonate present.

(*c*) *Volatile matter in carbon black*—After making due allowance for the foregoing considerations it might be expected that the weight of carbon dioxide absorbed by the soda-asbestos would bear a strict stoichiometric relationship to the weight of carbon black after the initial heating in nitrogen, and be proportional to the loss in weight during the oxidation stage. It might therefore appear unnecessary to utilise both the loss of weight in the oxidation stage and the weight of carbon dioxide absorbed, in order to arrive at a figure for the carbon black content. The influence of the volatile matter in the carbon black on the relationship between these values is, however, a significant one, and must be considered in some detail.

Volatile matter, in this connection, is the term given to the gases adsorbed at the surface of the carbon particles, which can be successively removed at rising temperature levels. These gases range from oxides of carbon that begin to come off at about 500° C. to the hydrocarbons and hydrogen, for the elimination of which temperatures up to 1400° C. are considered necessary by some workers. With channel blacks the proportion of such adsorbed gases may be considerable, frequently exceeding 6 per cent. of the total weight, but lamp blacks, acetylene black and most blacks produced by the furnace process have a low volatile content. The amount of volatile matter attached to any particular type of black is one of its most characteristic features and provides a ready means of differentiation between (for example) channel blacks and furnace blacks.

The evolution of volatile matter for the purpose of the present investigation has been studied at 600° and 900° C. Volatile matter evolved below 600° C. is lost to the atmosphere (together with decomposition products of the organic materials in the rubber compound), but must be allowed for when computing the weight of carbon black originally present. Since, however, the extent of this volatile fraction is not actually determined, it plays no direct part in identifying the black used. Of the volatile matter evolved over the temperature range 600° to 900° C., which forms the larger proportion of the total volatile matter, some will be oxidised to carbon dioxide and will be weighed in the soda-asbestos tube, whilst the remainder is non-oxidisable, and is represented by the discrepancy between the carbon as determined by the difference in weight before and after oxidation, and the carbon as determined from the carbon dioxide formed. This non-oxidisable fraction, although only a part of the total volatile matter, is accurately determinable, is characteristic of the type of black, and forms the basis used in this investigation for the differentiation between types. Its amount is calculated as follows.

If the total weight of dry carbon black originally present in a rubber compound is W, and a, b, c, d and e have the following meanings:

a = weight of volatile matter evolved below 600° C.,

b = weight of volatile matter evolved over the temperature interval 600° to 900° C., and not oxidisable to carbon dioxide,

c = weight of volatile matter evolved over the temperature interval 600° to 900° C., and oxidisable to carbon dioxide,

d = weight of non-volatile carbon,

e = weight of carbon dioxide produced,

then
$$W = a + b + c + d$$
and
$$0{\cdot}2729 \times e = c + d;$$
hence
$$W - a = b + 0{\cdot}2729\, e$$
or
$$b = (W - a) - 0{\cdot}2729\, e$$

Since the term $(W - a)$ represents the difference in weight before and after oxidation, the value of b can be readily calculated.

Many of the commoner carbon blacks used in the industry have been examined in this way and the data are presented in Table II.

It will be seen that the values of $100\, b/(W - a)$ in column (1) fall into two clearly defined groups, the high-volatile channel blacks, ranging from about 5 to 8 per cent., and the low-volatile furnace and lamp blacks with figures usually below 3 per cent.

When the type of carbon black used has been determined by reference to Table II, it is a simple matter to make a correction for the total volatile matter. Column (2) of Table II shows the ratio of oxidisable carbon (*i.e.*, that which is weighed as carbon dioxide) to original carbon black for the same series of standard grades, and allowance has been made for the volatile matter lost below 600° C. Again, it will be seen that a greater correction is necessary with channel blacks than with other types, although the values do not follow a strict relationship with the figures in column (1).

With binary mixtures of carbon blacks a useful estimate of the ratio of the two components can be obtained. It is, of course, necessary with mixtures to know the types of black present, and the confidence to be placed in the final result is dependent on the difference in the value of b for the two components. The value of b for the mixture determined experimentally will lie between the values for the two blacks considered separately according to the relative proportions of the blacks present. This ratio may be found from the following expression,

$$A = \frac{V - V_B}{V_A - V_B} \cdot 100,$$

in which

A = proportion of carbon black A calculated as a percentage of the total black present,
V = value of *b* for the mixture of blacks in the rubber as determined experimentally,
V_B = value of *b* for carbon black B } as given in Table II, column (1).
V_A = value of *b* for carbon black A } as given in Table II, column (1).

TABLE II

Column (1) shows values for non-oxidisable volatile matter evolved over the temperature range 600° to 900° C., calculated as a percentage of the carbon black after heating in nitrogen at 600° C.

Column (2) shows the proportion (per cent.) of true carbon in the original black calculated from the yield of carbon dioxide.

Sample	Type	(1) $\frac{b}{W - a}\,100$	(2) $\frac{0\cdot2729\,e}{W}\,100$
Micronex	Channel	6·0	91·6
Kosmobile H.M.	„	5·6	91·3
Spheron 4	„	5·3	93·8
Spheron 6	„	7·9	90·7
Spheron 9	„	5·7	91·7
Dixiedensed H.M.	„	6·7	90·8
Dixie R.I	„	5·2	91·5
Spheron C	„	4·5	93·6
Magecol	Lamp	0·2	97·4
Champion	„	0·8	98·1
Statex 93	Furnace	0·0	99·3
Statex K	„	3·4	96·0
Furnex	„	2·3	93·4
P.33	Thermal	0·7	96·5
Seval	„	2·3	97·1
Shawinigan	Acetylene	0·0	

RESULTS

In the tables that follow, results are given for a series of unvulcanised and vulcanised rubber compounds. The behaviour of the main types of carbon black in association with the more commonly used polymers has been studied, and mixings have been chosen to illustrate the influence of whiting and china clay. All samples of carbon black were dried at 120° C. for 4 hours before mixing into rubber.

The results were calculated in the manner already outlined, but for the sake of further clarity intermediate stages in the calculation have been included in columnar form.

Although the black loadings are nominal, care was taken to reduce as far as possible the errors often associated with small-scale compounding.

With the unvulcanised compounds, somewhat greater variation than seemed desirable occurred in the pale crepe series. The carbon black content in these abnormal cases was checked by the nitric acid decomposition method and the results confirmed this variation, indicating that lack of uniformity in the compound was responsible. Thereafter, a stricter mixing technique was followed, and subsequent results showed a higher degree of precision.

The black loadings in the vulcanised compounds, nominally 15 per cent., were actually obtained by check-weighing, and the experimental results show a degree of accuracy, even in presence of china clay and calcium carbonate, that compares favourably with that given by the nitric acid decomposition method. Unfortunately the assessment of accuracy by direct comparison with laboratory mixed compounds is not valid beyond a certain point, owing to the difficulty of preparing compounded rubber whose actual composition is itself beyond all doubt.

TABLE III

UNVULCANISED CHANNEL BLACK COMPOUNDS

(Micronex; $\frac{0{\cdot}2729\, e}{\mathrm{W}}\, 100 = 91{\cdot}6$)

Polymer	(a)	(b)	(c)	(d)	(e)
Pale crepe	30	0·1026	0·1050	0·0002	30·2
	30	0·1014	0·1035	0·0002	30·2
	15	0·1186	0·0607	0·0002	15·0
	15	0·1048	0·0532	0·0002	14·9
	7·5	0·1134	0·0278	0·0003	7·0
	7·5	0·1040	0·0254	0·0003	7·0
	3·75	0·1168	0·0148	0·0003	3·5
GR–S	15	0·1250	0·0640	0·0003	15·0
	15	0·1774	0·0907	0·0004	15·0
	7·5	0·1263	0·0326	0·0003	7·4
	7·5	0·1190	0·0309	0·0003	7·5
GR–I	15	0·1212	0·0615	0·0001	15·0
	15	0·1447	0·0734	0·0001	15·0
	7·5	0·1094	0·0279	0·0001	7·5
	7·5	0·1170	0·0297	0·0001	7·5
Perbunan	15	0·1121	0·0616	0·0014	15·0
	15	0·1008	0·0555	0·0013	15·0
	7·5	0·1303	0·0394	0·0018	7·5

TABLE IV

UNVULCANISED LAMP BLACK COMPOUNDS

(Magecol; $\frac{0{\cdot}2729\, e}{\mathrm{W}}\, 100 = 97{\cdot}4$)

Polymer	(a)	(b)	(c)	(d)	(e)
Pale crepe	30	0·1071	0·1140	0·0002	29·6
	30	0·1018	0·1086	0·0002	29·7
	15	0·0777	0·0417	0·0002	14·8
	15	0·0893	0·0478	0·0002	14·8
	7·5	0·0840	0·0231	0·0002	7·4
	7·5	0·0920	0·0249	0·0002	7·3
	3·75	0·0744	0·0107	0·0002	3·83
	3·75	0·0592	0·0085	0·0002	3·75
Smoked sheet ..	15	0·1258	0·0676	0·0002	14·9
	15	0·0954	0·0514	0·0002	15·0
	7·5	0·1260	0·0341	0·0003	7·4
	7·5	0·1321	0·0359	0·0003	7·4
GR–S	15	0·1136	0·0609	0·0002	14·8
	15	0·2038	0·1100	0·0004	14·9
	7·5	0·1042	0·0284	0·0002	7·4
	7·5	0·1026	0·0279	0·0002	7·4
GR–I	15	0·1326	0·0709	0·0001	14·9
	15	0·1269	0·0680	0·0001	14·9
	7·5	0·1130	0·0303	0·0001	7·4
	7·5	0·1200	0·0321	0·0001	7·4
Perbunan	15	0·1040	0·0603	0·0014	14·9
	15	0·1108	0·0643	0·0014	14·9
	7·5	0·1115	0·0354	0·0016	7·5
	7·5	0·1200	0·0381	0·0017	7·5

KEY TO TABLES III AND IV—

Column	(a)	Nominal carbon black loading, per cent.
„	(b)	Weight in grams of the sample taken for test.
„	(c)	Weight of CO_2 obtained.
„	(d)	Correction for carbonaceous residue from the polymer as determined by consulting Table I.
„	(e)	Carbon black found, per cent.

With binary mixtures of carbon black, substitution of the appropriate values for V_A and V_B in the formula $A = 100 (V - V_B)/(V_A - V_B)$ requires a knowledge of the type and brand of the two separate constituents, and since such data are unlikely to be available for compounded rubbers of unknown origin, the method of evaluating mixtures can usually be applied with confidence only to routine control analysis where the qualitative nature of the carbon black is not in doubt. The calculated values of A are very sensitive to the substituted values of V_A and B_B, and experience has shown that it is unwise to substitute even the mean values for each class of black, as values of A obtained in this way may vary up to about 20 per cent.

TABLE V

VULCANISED COMPOUNDS

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Smoked sheet	67·5	77·5	77·5	67·5	—	—	—
GR–S ..	—	—	—	—	77·5	—	—
Perbunan	—	—	—	—	—	74·0	74·0
Sulphur ..	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Stearic acid	1·0	1·0	1·0	1·0	1·0	2·0	2·0
Mercapto-benzthiazole	1·0	1·0	1·0	1·0	1·0	1·5	1·5
Zinc oxide	3·0	3·0	3·0	3·0	3·0	5·0	5·0
China clay	10·0	—	—	—	—	—	—
Calcium carbonate	—	—	—	10·0	—	—	—
Furnace black (Statex 93)	14·5	—	—	—	—	—	—
Lamp black (Champion)	—	—	15·2	—	—	—	—
Acetylene black	—	14·5	—	—	—	—	—
Channel black (Spheron 9)	—	—	—	15·8	—	—	—
Channel black (Kosmos H.M.)	—	—	—	—	15·8	—	—
Furnace black (Statex K)	—	—	—	—	—	16·2	—
Channel black (Spheron C)	—	—	—	—	—	—	16·5
Carbon black by experiment	14·3	14·0	15·3	15·5	16·0	16·6	16·9
Value of $\frac{b}{W - a} \cdot 100$	0	0	0·23	5·8	5·6	3·2	4·4

When the precise grades of carbon black are known, however, and the appropriate values of V_A and V_B can be substituted, a useful estimate of the ratio can be obtained. Thus, for a mixing containing Micronex ($V = 6{\cdot}0$) and Magecol lamp black ($V = 0{\cdot}2$), the value of V determined experimentally for the mixture was 3·7. Substituting these values in the formula, the value of A becomes 40 per cent., indicating a 40 : 60 lamp black - channel black mixture. Such good agreement, however, is regarded as fortuitous and a degree of accuracy within ±5 per cent. even in favourable circumstances is not claimed.

In a second example it was known that a mixture of Micronex and Statex K furnace black had been incorporated in a rubber mixing; it was required to find the ratio of these constituents. The following values were substituted in the formula:

V_A	3·4
V_B	6·0
V	4·6 (determined experimentally)

The value of A is calculated as 46 per cent., which suggests a mixture of approximately equal parts of Micronex and Statex K. For reasons stated previously, however, the limits of variability of the ratio might reasonably be expected to range from 40 to 50 per cent. of Statex K.

DESCRIPTION OF METHOD

REAGENTS REQUIRED—

(1) "Oxygen-free" or "pure" nitrogen.

(2) Oxygen.

(3) Alkaline pyrogallol solution—Dissolve 50 g. of pelletised caustic potash in 100 ml. of water and dissolve 30 g. of pyrogallol in the solution.

(4) Xylene.

(5) Concentrated sulphuric acid.

(6) Magnesium perchlorate (Anhydrone).

(7) Soda-asbestos, Carbosorb, 14 to 20 mesh.

(8) Acidified potassium permanganate solution—Dissolve potassium permanganate crystals in the minimum volume of water and dilute with concentrated sulphuric acid to give a 0·02 *N* solution.

(9) Cupric oxide
(10) Platinised asbestos
(11) Ignited Gooch asbestos
} for combustion tube packing.

(12) Copper turnings reduced *in situ* with hydrogen.

APPARATUS—

Nitrogen supply—"Oxygen-free" nitrogen is fed from a cylinder, through a flowmeter (not shown on diagram) at the rate of 500 ml. per minute. It is passed then through two Drechsel gas-washing bottles, A and B, containing alkaline pyrogallol solution, a third Drechsel bottle, C, containing concentrated sulphuric acid and a 30-cm. column of reduced copper turnings heated by a small furnace, D. The purified gas is admitted to the combustion tube via a T-piece, F, through which passes a silica rod, H, with a hooked end of nickel-chromium wire for manipulating the combustion boat. G is a trap for collecting condensible vapours formed during the pyrolysis and E is the final absorption vessel containing xylene. Residual gases are drawn away through a small funnel attached to a filter pump.

For the oxidation train oxygen is taken from a cylinder and purified by passing through two U-tubes, J and K, containing Anhydrone and soda-asbestos respectively. As in the first tube, the boat is manipulated by means of a silica rod sliding in a glass T-piece. The combustion tube is packed in the positions shown on the diagram with (*a*) platinised asbestos and (*b*) cupric oxide, with intermediate and supporting packings of Gooch asbestos.

L is an Arnold bubbler containing acidified potassium permanganate, and after passing through this and a U-tube, M, containing Anhydrone, the effluent gases are passed through a U-tube, N, containing soda-asbestos, in which the carbon dioxide is absorbed. The train is terminated by an Arnold bubbler, O, containing concentrated sulphuric acid which serves to indicate the rate of oxygen flow.

PROCEDURE—

Samples are given a prior extraction with acetone and, if mineral rubber is thought to be present, chloroform extraction is necessary also, these operations being carried out by standard methods.[11]

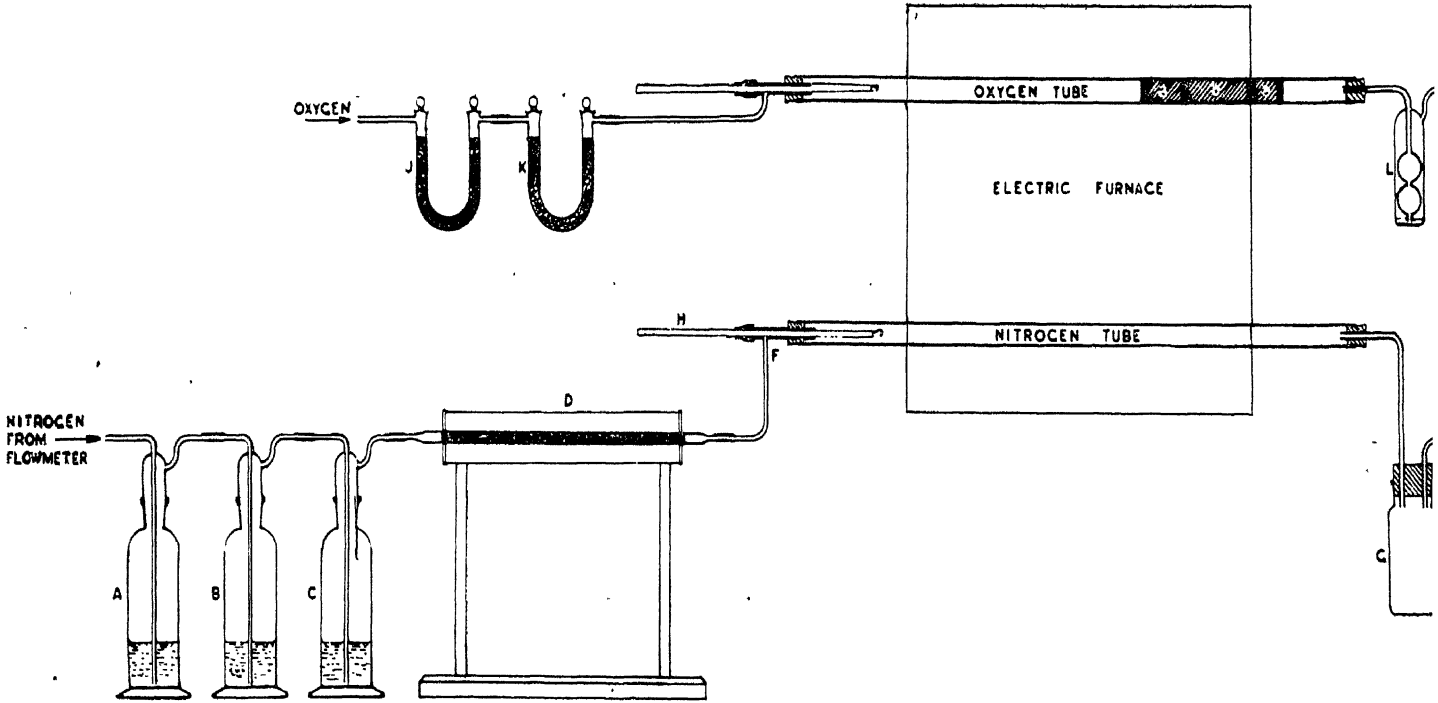
Weigh accurately an amount of sample equivalent to approximately 0·1 g. of the original sample into a silica combustion boat (5 cm. by 1 cm.) and place just inside the cool end of the nitrogen combustion tube which is then closed with the entry fitting. Pass nitrogen into the train at the rate of 500 ml. per minute, switch on the preheater furnace, D, and the main electric furnace and allow them to reach temperatures of 500° and 600° C. respectively. When these temperatures have been attained push the combustion boat slowly forward by means of the silica rod, until in 5 minutes it reaches the hot zone of the furnace, where it is heated for a further 5 min. Then return the boat to its original position at the end of the tube, cool for 10 minutes with nitrogen still passing, and weigh if it is required to determine the type of black or the proportions of black present in a binary mixture.

Now transfer the boat and contents to the oxygen tube, to which oxygen is admitted at the rate of 25 ml. per minute, and when the temperature of the furnace has reached 900° C., push the boat into the hot zone for 15 minutes. Disconnect from the train the soda-asbestos absorption tube, which has previously been weighed, and re-weigh, and calculate the corrected figure for carbon dioxide as already described under "Calculation of Results."

After the absorption tube has been disconnected remove the entry fitting, take out the boat, cool in a desiccator and weigh. The loss of weight of the boat and its contents on oxidation is equal to (W − *a*) in the expression $0{\cdot}2729\,x/(W - a)$.

SUMMARY

The method described in this paper is based on the removal of volatile organic ingredients of the compound by heating in a current of purified nitrogen and, after reweighing if desired, subsequent combustion of the carbon black to carbon dioxide, which is collected and weighed. The effect of the adsorptive nature of the carbon black has been studied, and the volatile constituents of many of the standard grades of carbon black used in the industry have been determined under the experimental conditions used.



KEY TO DIAGRAM

A, B	Alkaline pyrogallol wash-bottles	J	U-tube containing anhydrone
C	Concentrated sulphuric acid wash-bottle	K	U-tube containing soda asbestos
D	Heater for reduced copper column	L	Acidified permanganate wash-bottles
E	Absorption bottle containing xylene	M	U-tube containing anhydrone
F	T-piece entry tube	N	U-tube containing soda-asbestos
G	Bottle for trapping effluent gases	O	Sulphuric acid bubbler
H	Silica rod for manipulating boat		

The replacement of the arbitrary factor used in earlier methods by the determination of the volatile matter in the carbon black not only allows a more accurate correction to be applied to the quantitative determination of carbon, but also provides an indication of the type of carbon black used in a rubber compound. Where a mixture of high- and low-volatile carbon blacks has been used the method gives a useful estimate of the ratio of the two types.

The authors wish to express thanks to Dr. Davey for helpful suggestions in connection with the work, and to the Technical Director of the Dunlop Rubber Company Limited for permission to publish this paper.

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THE DUNLOP RUBBER COMPANY
ERDINGTON, BIRMINGHAM

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Rapid Determination of Carbon Dioxide in Absorbent Liquors or other Carbonate Solutions

BY B. E. DIXON AND R. A. WILLIAMS

IN the course of experimental work on alkaline absorbent liquors a requirement arose for a robust, self-contained and simple apparatus for use in the rapid and accurate determination of carbon dioxide. Several methods for the estimation of carbon dioxide have been described that depend on the measurement of the gas pressure developed on treatment of carbonate by acid,[1,2,3] etc. Most of these methods, however, involve the use of one or more instruments or laboratory services such as thermostats, balances, barometers, vacuum pumps, a supply of carbon dioxide or a source of heat. The ease of operation of the method in its simplest form is so attractive that an attempt was made to improve the accuracy by a study of the inherent sources of error. The apparatus now described can be used for the estimation of carbon dioxide in absence of the usual laboratory facilities.

APPARATUS

The reaction vessel (Fig. 1) was constructed in stainless steel, which permitted the rapid attainment of a constant temperature in the gaseous phase by quick dissipation of the heat of reaction. The alloy used, "Staybrite FDP," is completely inert towards both phosphoric acid (20 per cent. v/v) and the bases used, under the conditions of operation. The vessel is a squat cylinder having a flat lid fitted with an exit tube and a thermometer. The lid is secured to the vessel by means of six swing bolts and lugs, and the apparatus made gas-tight by means of a smooth rubber washer between lid and cylinder. The base of the vessel is divided into two compartments by two planes inclined at a gentle slope. The exit tube is connected to a Y-piece, one end of which is attached to a tap, the other end leading to a manometer. The cubic content of the reaction vessel and of the connection as far as the level of mercury in the manometer is measured.

PROCEDURE

The reaction vessel may be used (A) with an open manometer or (B), if a vacuum pump is available, with a closed manometer. An accurate mechanical pressure gauge can replace the manometer in certain circumstances (see below).

(A) WITH AN OPEN MANOMETER—

Five ml. of phosphoric acid (20 per cent. v/v) are measured into one compartment and 5 ml. of the sample liquor (containing not more than 1 g. of base or 0·4 g. of combined CO_2) into the other compartment. The lid is carefully secured by means of the bolts and the tap on the Y-piece closed. The initial temperature is taken. The vessel is now tilted gradually

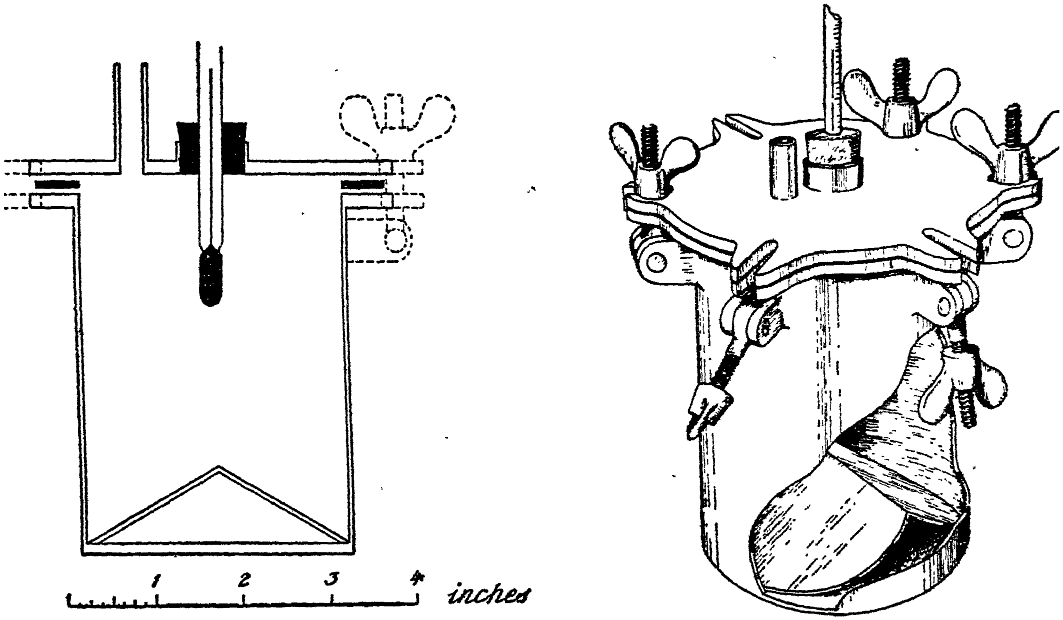

Fig. 1

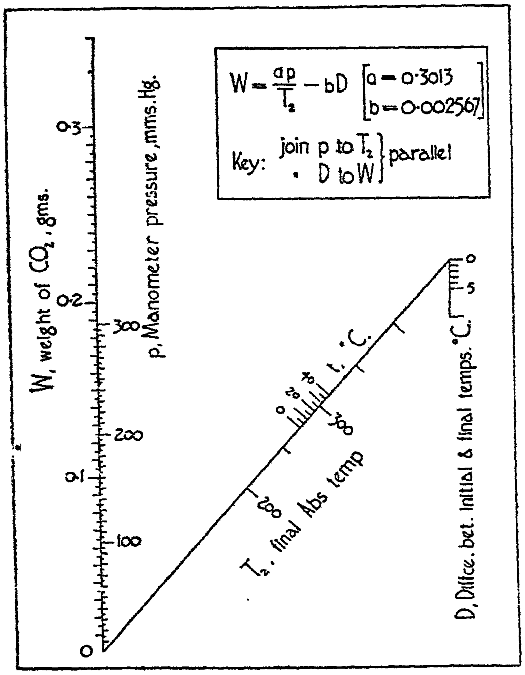

Fig. 2

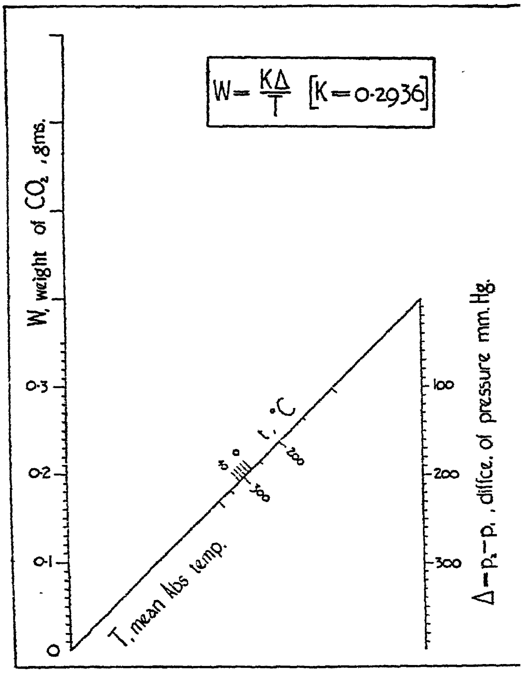

Fig. 3

to mix the acid and base and the mixture finally thoroughly roused by vigorous shaking. After about 1 minute the column of mercury becomes steady and the manometer pressure and final temperature are taken.

(B) WITH A CLOSED MANOMETER—

The open manometer in (A) is replaced by a closed manometer and a vacuum pump is attached to the tap on the Y-piece. The acid and base are added, the lid secured and the initial temperature taken as in (A). The vessel is then evacuated to a pressure of about 10 mm. of mercury as shown by the difference in level of the manometer columns. The pump is then cut out of circuit by means of the tap and the liquids mixed as before. The difference in manometer levels and the final temperature are noted.

The weight of carbon dioxide is then read directly from the graphs (Fig. 2 for Procedure (A), or Fig. 3 for Procedure (B)).

EQUATIONS AND GRAPHS

(A) WITH AN OPEN MANOMETER—

The weight in grams of carbon dioxide obtained in Procedure (A) on the assumption that no change occurs in the barometric pressure or in the volume of the gases is given by the expression:

$$\mathrm{W} = \left(\frac{\mathrm{V} \times 0{\cdot}001965 \times 273}{760}\right) \times \left(\frac{\mathrm{P} + p}{\mathrm{T}_2} - \frac{\mathrm{P}}{\mathrm{T}_1}\right) \quad \ldots \quad \ldots \quad \text{(i)}$$

which can be expressed in the form

$$\mathrm{W} = a_1\left(\frac{p}{\mathrm{T}_2} - \frac{\mathrm{PD}}{\mathrm{T}_1\mathrm{T}_2}\right) \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(ii)}$$

Equation (ii) can with no appreciable loss of accuracy be simplified to

$$\mathrm{W} = \frac{a_1 p}{\mathrm{T}_2} - b\mathrm{D} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(iii)}$$

where a_1 and b are constants.

By substituting in equations (i) and (iii) imaginary values for pressures and temperatures within the working range, it was found that the deviation never exceeded a few tenths of a milligram. For practical purposes, therefore, equation (iii) can be substituted for equation (i).

There are two small sources of error for which correction must be made in the above equations, *viz.* (*a*) the amount of carbon dioxide dissolved in the mixed liquids, and (*b*) the volume of gas displacing the mercury in the manometer at the end of the reaction. Both these quantities are functions of p, and hence proper correction factors may be incorporated in the constant a_1 in equation (iii) to give the working equation,

$$\mathrm{W} = \qquad - b\mathrm{D} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(iv)}$$

the nomograph corresponding to (iv) is shown in Fig. 2.

(B) WITH A CLOSED MANOMETER—

In this case the weight of carbon dioxide is given by

$$\mathrm{W} = \left(\frac{\mathrm{V} \times 0{\cdot}001965 \times 273}{760}\right) \times \left(\frac{p_2}{\mathrm{T}_2} - \frac{p_1}{\mathrm{T}_1}\right) \quad \ldots \quad \ldots \quad \ldots \quad \text{(v)}$$

which can be simplified to

$$\mathrm{W} = \frac{\mathrm{K}_1\Delta}{\mathrm{T}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(vi)}$$

Here again, for values of T, T_1, p_1, etc. within the working range, equations (v) and (vi) give practically identical values for W.

The slight changes in temperature and volume of the gas during the reaction are insignificant as sources of error in Procedure (B), but a correction must be made as in Procedure (A) to offset the solubility of carbon dioxide in the mixed liquids.

The nomograph corresponding to the working equation (vii) is shown in Fig. 3.

$$\mathrm{W} = \frac{\mathrm{K}\Delta}{\mathrm{T}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \text{(vii)}$$

If a mechanical pressure gauge is used in place of a manometer in Procedure (B), the method is unchanged and the results can be read off on Fig. 3. In Procedure (A), however, the manometer cannot be replaced by a pressure gauge if the gauge is of such a type that the gases enclosed in the instrument change appreciably in volume.

SYMBOLS

W = Weight of carbon dioxide in g.
V = Volume of gases (ml.) in reaction vessel and connection.
T_1 = Temperature (abs.) of gases before reaction.
T_2 = Temperature (abs.) of gases after reaction.
D = $T_2 - T_1$.
p = Manometric pressure (mm. of mercury).
P = Barometric pressure (mm.), which in practice is unchanged during the determination.
$a_1 = \dfrac{V \times 0{\cdot}001965 \times 273}{760}$
b = $760 \times a_1/(293)^2$. The second term in equation (ii) is relatively small and P can be equated to 760 and T_1T_2 to $(293)^2$, the average temperature of operation being taken as 20° C.
a = $F_1F_2\,a_1$, where F_1 is the volume correction factor and F_2 the solubility correction factor. F_1 is calculated from the dimensions of the manometer tube, which cannot conveniently be much less than 5 mm. in diameter. The solubility of carbon dioxide in the mixed liquids (*viz.*, 5 per cent. phosphoric acid partially neutralised with up to 3 per cent. of base) was found to be approximately 0·01 g. CO_2 per 10 ml. at 760 mm. partial pressure of CO_2.
p_1 = Manometric pressure (mm.) before reaction.
p_2 = Manometric pressure (mm.) after reaction.
Δ = $p_2 - p_1$.
T = Mean temperature (abs.) of gases during reaction.
K_1 = a_1.
K = F_2a_1.
a, b and K are apparatus constants.

RESULTS

Results obtained with this apparatus, using Procedures (A) and (B), are shown in the table.

Absorbent solution, 5 ml.	CO_2 theoretical, mg.	CO_2 determined by other methods mg.	CO_2 found, mg.	
			Procedure (A)	Procedure (B)
Sodium carbonate	320·5	318·5 (1)	318	
	194		195	
	172·5			172·5
	124			125·5
	86		84	85
	43		41	43
Monoethanolamine		122 (1)		122·5
		112 (1)	112·5	
Triethanolamine		71 (1)	71	71
		35·5 (1)	34	36
Ammonium carbonate		197·5 (2)	200	198
Ethylenediamine		66·5 (1)	66	69

(1) By addition of barium chloride followed by separation of the precipitated barium carbonate, which is dissolved in acid and titrated back with alkali.
(2) By Schrötter apparatus.

The method has been described for use with liquid samples. Solid water-soluble or acid-soluble carbonates can also be determined provided that water is added to give a total bulk of 5 ml. in the compartment containing the base.

SUMMARY

A simple and robust apparatus of stainless steel is described for the rapid determination of carbon dioxide in liquid absorbents or other carbonate solutions. The carbon dioxide is

generated by the action of cold dilute acid and from the pressure and temperatures recorded the weight of gas is read off directly from a graph. By the use of simplified expressions for the weight of carbon dioxide embodying corrections for significant sources of error it has been possible to retain a simple technique and apparatus design without sacrifice of accuracy.

We wish to thank the Government Chemist for permission to publish this work.

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Notes

THE COLORIMETIRIC DETERMINATION OF SMALL QUANTITIES OF ALUMINIUM

A recent abstract[1] describes a method by Mervel for the extraction of the aluminium 8-hydroxyquinoline complex with benzene and implies that iron and certain other interfering elements may be removed by precipitation with sodium hydroxide.

In these laboratories we have observed that the removal of iron by precipitation is invariably incomplete, even in the absence of tungsten. Gentry and Sherrington,[2] in their very comprehensive treatment of the subject, do not recommend precipitation for the separation of iron and their method, which employs conversion of iron to ferrocyanide, successfully eliminates interference. They also suggest[2] that mercury cathode electrolysis may be used for the removal of many of the heavy metals before determination of aluminium. However, we have observed that if this latter technique is adopted sufficient iron still remains in solution after electrolysis to interfere with the aluminium determination, and this iron must be removed by a preliminary extraction at pH 2·0 to 2·5 before the pH is raised to 5·0 for extraction of the aluminium complex.

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THE SEPARATION OF BERYLLIUM, ALUMINIUM, URANIUM AND VANADIUM BY CUPFERRON IN SYSTEMATIC QUALITATIVE ANALYSIS

In a modified scheme of analysis, based on that of Noyes and Bray,[1] embracing the commoner of the "rarer' elements, it is necessary to separate in Group III the ions $Fe^{\cdot\cdot\cdot}$, $Cr^{\cdot\cdot\cdot}$, $Al^{\cdot\cdot\cdot}$, WO_4'', VO_4''', PO_4''', $Ti^{\cdot\cdot\cdot\cdot}$ $Zr^{\cdot\cdot\cdot\cdot}$, $Ga^{\cdot\cdot\cdot}$, $Mn^{\cdot\cdot}$, $Zn^{\cdot\cdot}$, $Co^{\cdot\cdot}$, $Ni^{\cdot\cdot}$, $UO_2^{\cdot\cdot}$, $Be^{\cdot\cdot}$, $Ce^{\cdot\cdot\cdot}$ and $Th^{\cdot\cdot\cdot\cdot}$. Following the usual scheme, this group is subjected to a basic acetate separation followed by treatment with sodium peroxide, yielding a filtrate containing CrO_4'', AlO_2', WO_4'', PO_4''', VO_4''', $HZnO_2'$, BeO_2'' and peruranate, *e.g.*, UO_8''''. This filtrate is treated under pressure with sodium bicarbonate, leaving a residue that may contain beryllium, aluminium, uranium, vanadium and zinc. The zinc is removed by an ammonia precipitation.

The separation of the remaining elements is tedious and involves evaporation to basic acetates, chloroform extraction and the use of several reagents. It was thought that the process could be simplified by the use of cupferron.

The following scheme was developed for use on the semi-micro scale.

The residue from removal of zinc will contain $Al(OH)_3$,$Be(OH)_2$, $(UO_2)_3(VO_4)_2$,$AlVO_4$ and uranium occluded by the alumina.

<table>
<tr><td colspan="4"><i>Residue</i>: Dissolve in 0·5 ml. of conc. HCl, add 2·5 ml. of water, excess of 6% aqueous cupferron and macerated filter paper, shake well, centrifuge, wash with 10% HCl containing a few drops of cupferron solution</td></tr>
<tr><td rowspan="3"><i>Residue</i>: V and Fe. Dark red ppt. shows V. Evaporate to dryness with conc. HNO_3 to destroy organic matter. Dissolve in 10% H_2SO_4, add 1% $KMnO_4$ solution until faint pink, then 2 drops of H_2O_2. Red colour confirms V</td><td colspan="3"><i>Filtrate</i>: Add 0·5 g. of sodium acetate, boil, centrifuge and wash</td></tr>
<tr><td rowspan="2"><i>Residue</i>: Al. Destroy organic matter with HNO_3; confirm Al by alizarin or morin test</td><td colspan="2"><i>Filtrate</i>: Destroy organic matter with HNO_3, take up in few drops of dil. HCl, add slight excess of $(NH_4)_2CO_3$ solution, boil and centrifuge</td></tr>
<tr><td><i>Residue</i>: Be. Confirm by quinalizarin</td><td><i>Filtrate</i>: Add H_2O_2—orange-yellow colour; or acidify and add $K_4Fe(CN)_6$ solution—dark brown ppt. shows U</td></tr>
</table>

This process has been tested on the macro, semi-micro and micro scales by proportional alteration of quantities and found satisfactory in all combinations of ions.

The degree of separation has been tested by classical methods and by microchemical tests and would appear to be quantitative within the limits of these tests. Each precipitate, after washing, appears to be free from contamination.

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September, 1948

THE PREPARATION OF N-PHENYLANTHRANILIC ACID INDICATOR SOLUTIONS

THE generally recommended method of preparing N-phenylanthranilic acid indicator solutions with sodium carbonate[1] is somewhat tedious. Furthermore, the solution quickly darkens in colour, and the sensitivity decreases slightly on keeping. More objectionable is the rapid growth of a fungoid organism, possibly owing to the relationship of the indicator molecule to the well known plant growth factors. The metabolic products of this organism do not seem to affect the properties of the solution, but may contribute in some way to decreasing sensitivity. In six instances examined, the organism was *Aspergillus niger*, probably air-borne, or carried in the distilled water.

It was thought that it would be simpler and more convenient to prepare the solution with the theoretical amount of sodium hydroxide, and that mould growth might be inhibited by a suitable preservative.

Of the solutions studied, the following were worthy of note.

(*a*) 0·005 *M* Indicator in 0·0125 *M* sodium carbonate[1]; 0·535 g. of the indicator was boiled with 30 ml. of water containing 0·50 g. of sodium carbonate and diluted to 500 ml. The substance dissolves with difficulty.

(*b*) For this solution, 0·25 g. of the indicator was warmed with 12·0 ml. of 0·1 *N* sodium hydroxide and diluted to 250 ml. (0·1 per cent. solution). The substance dissolves readily.

(*c*) As in (*b*) but with addition of 0·10 g. of thymol to the sodium hydroxide solution.

(*d*) As in (*b*) but with 1 ml. of chloroform added.

These solutions have been in use for 18 months, stored out of contact with direct sunlight, but intermittently exposed during use. Solution (*a*) rapidly turned brown in colour and in a few weeks had a heavy mycelial growth of *Asp. niger*. Solutions (*b*), (*c*) and (*d*) remained water-white for 5 or 6 months and then gradually became very slightly discoloured, the order of increasing intensity being (*b*), (*c*), (*d*). Mould growth did not begin until 5 or 6 months had elapsed, and after 18 months (*c*) was practically clear, (*b*) had a very slight growth, and in (*d*) the growth was about one-eighth of that in (*a*). In each the mould was *Aspergillus niger*. All solutions suffered to a varying degree an initial slow decrease in sensitivity, or in speed and quality of response, and maintained an order of increasing sensitivity of (*a*), (*c*), (*b*), (*d*) throughout. The indicator correction remained constant for each solution during the period of test, and may be shown by the following figures obtained in ferrous iron titrations by 0·1 *N* potassium dichromate at an acidity of 4 *N* sulphuric acid, using 1·0 ml. of indicator 18 months old:

Indicator solution	(*a*)	(*b*)	(*c*)	(*d*)	Theory
Mean titration, ml.	24·18	24·19	24·21	24·19	24·11

It appears that the use of sodium hydroxide as a solvent is advantageous, and that whilst chloroform appears in some way to catalyse the indicator response, it is ineffective in reducing mould growth and increases colour formation in comparison with that in the simple sodium hydroxide solution. Thymol is effective in preventing mould growth, but introduces a small error by consuming oxidant, and has a slightly adverse effect on the quality of the end-point.

Figures obtained have shown that the indicator correction with N-phenylanthranilic acid is not so negligible as was formerly assumed. If the titration is taken to the first permanent colour change, the correction amounts to 0·04 ml. of 0·1 *N* oxidant per 0·5 ml. of 0·005 *M* or 0·1 per cent. indicator solution; or if the titration is continued to the first development of the purple colour (requiring a waiting time of 5 minutes) the correction is 0·06 ml. of 0·1 *N* oxidant per 0·5 ml. of indicator solution. Addition of phosphoric acid reduces the correction slightly, but renders the end-point more sluggish.

It is recommended that the indicator be prepared by method (*b*) above and that, in titrations, 5 minutes be allowed after each of the last 3 drops of the titration. The indicator solutions appear to be stable and active for long periods.

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THE HYDROLYTIC EVALUATION OF CELLULOSE

THE methods for the quantitative estimation of cellulose are divisible into two groups. The first regards the cellulose as the main structural material of plants after protein, starch, fat, lignin, etc., have been removed. The second group dates from the demonstration by Monier-Williams[1] and others, that cellulose could be almost completely converted to glucose by hydrolysis with strong sulphuric acid.

In the hydrolytic methods, the "cellulose" is calculated from the reducing value of the hydrolysate as "glucose anhydride." The reaction is represented, $(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$, and as practically quantitative conversion to the monosaccharide has usually been assumed, the theoretical factor of 0·90 is used to convert the estimated dextrose to cellulose.

During a recent investigation on the isolation of plant celluloses, the hydrolytic method of Waksman and Stevens[2] has been examined for use in evaluating "purified" celluloses. For the sake of convenience, half the quantities recommended by them were employed. Between 0·3 and 0·4 g. of cellulose was weighed directly into a light 250-ml. conical flask with a standard joint. Five ml. of 80 per cent. sulphuric acid were added, and the mixture allowed to stand for 2½ hours with frequent stirring, and then diluted with 75 ml. of cold water, added rapidly to prevent charring, and boiled under reflux for 5 hours. The liquid was cooled, neutralised with caustic soda solution (using Johnson's Universal indicator paper), filtered through a small Buchner funnel and made up to 200 ml.

The dextrose of the hydrolysate was determined by the Jackson[3] modification of the Lane and Eynon method,[4] using 10 ml. of the mixed Fehling - Soxhlet solution.

The effect of slight variations in concentration and volume of acid employed in the hydrolytic procedure was studied, using Whatman Filter Paper, Grade 42 (α-cellulose 98·8 per cent., copper number 0·15, ash 0·012 per cent., pentosan content 0·54 per cent.), which had been cut into small pieces and dried over sulphuric acid *in vacuo.*

The results are shown in Table I.

TABLE I

Paper taken, g.	Strong acid composition		Hydrolysate titre ml.	Factor (Lane and Eynon)	Corrected factor (Jackson modification)	mg. Dextrose per 100 ml. mg.	Cellulose found g.	Cellulose recovery %
	80% H_2SO_4 ml.	H_2O ml.						
0·5130	5·0	0·0	19·5	49·4	50·39	258·4	0·4651	90·7
0·3582	4·8	0·2	28·0	50·0	51·00	182·1	0·3278	91·5
0·4150	4·8	0·2	23·9	49·8	50·80	212·6	0·3827	92·2
0·4490	5·0	0·1	21·8	49·6	50·59	232·1	0·4178	93·1
0·4060	10·0	0·0	28·7	50·0	51·00	177·7	0·3199	78·8
0·3390.	10·0	0·0	34·8	50·4	51·41	147·7	0·2659	78·2

The recovery of 90·7 per cent. by the Waksman technique shows that the hydrolytic procedure does not result in the complete conversion of the polysaccharide to the monosaccharide, and that the theoretical factor of 0·90 for the conversion of the dextrose found to cellulose is unsuitable. The low recovery may be due either to incomplete hydrolysis, or to degradation of the products of hydrolysis by the strong acid.

With 10 ml. of 80 per cent. sulphuric acid, the recovery is about 11 per cent. lower still, suggesting that hydrolysis has proceeded as far as possible, but that the degradation of the resulting products is increased. The same conclusion is inferred from the effect of dilution of the strong acid, which is seen to favour the greater recovery of dextrose.

The procedure described above has been standardised, using a purified cotton cellulose, to obtain an arbitrary factor for assessing the recovery of cellulose from the hydrolysate (*cf.* determination of pentosans).

Purified Texas cotton was thoroughly extracted with chloroform, shaken with 0·1 *N* hydrochloric acid for 2 hours, washed with distilled water until the washings were neutral to bromocresol purple, and partially dried over concentrated sulphuric acid *in vacuo.* It was stored in a stoppered bottle. Its moisture content was 1·51 per cent. and it contained no ash.

The results obtained with this pure cellulose are presented in Table II.

TABLE II

Cotton taken, g.	Moisture-free cotton, g.	Titre, ml.	Corrected factor	Dextrose per 100 ml. mg.	Cellulose found, g.	Cellulose recovery, %
0·3653	0·3598	28·4	51·00	179·6	0·3233	89·86
0·5130	0·5052	19·6	50·39	257·1	0·4628	91·62
0·4158	0·4095	24·6	50·80	206·5	0·3717	90·77
0·4754	0·4682	21·4	50·49	235·9	0·4246	90·69

The mean recovery is 90·73 ± 0·45 per cent. (standard error of mean).

The factor to convert dextrose to cellulose is therefore,

Cellulose = Dextrose × 0·99.

The factor 0·99 is suggested for use with the technique given above, provided the limitations of the use of an arbitrary factor, and of the method itself generally, are clearly understood.

On application to Whatman filter paper, Grade 54 (α-cellulose 99·3 per cent., copper number 0·23, ash 0·025 per cent., pentosan content 0·59 per cent.), the method gave a recovery of 100·25 ± 0·25 per cent., indicating the applicability of the suggested factor to the evaluation of purified celluloses.

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AN INTERNAL INDICATOR FOR ARSENITE - HYPOCHLORITE TITRATIONS

We have carried out some tests by the modified Penot method described by Sinn[1] which uses quinoline yellow as an indicator. We have not been able to obtain satisfactory results; the indicator did not change colour at all during the titration. This may be due to differences in the dyestuffs used as indicators.

We have, however, found that our quinoline yellow is a very satisfactory internal indicator for the classical Penot method for the titration of hypochlorite with sodium arsenite. It is much more convenient than the use of starch iodide paper as an external indicator. We have used it for the analysis of hypochlorite bleach liquors for some time past and the results always agree well with check tests using starch iodide indicator paper. There is only one small precaution to be observed—the quinoline yellow indicator must be added shortly, say 2 or 3 ml., before the end-point. The colour change is sharp, colourless to yellow-green.

By means of this internal indicator the great disadvantage of the Penot method—the use of an external indicator—is overcome and the method becomes as convenient as the iodide - thiosulphate method and, of course, very much more economical in reagents.

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December, 1948

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Analysis of Sulphadiazine and Sulphathiazole Mixtures. D. Barnes (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 653–655)—This simple, rapid spectrophotometric method for the analysis of mixtures containing sulphadiazine and sulphathiazole is based on the observation that, in strongly acid solution, sulphadiazine shows a peak absorption at 239 mμ., which diminishes to a low flat region at 270 to 300 mμ., whilst sulphathiazole has a maximum at 278 mμ. and a minimum at 240 mμ.

Procedure—Dissolve a convenient quantity of the sample by thorough shaking in 200 ml. of 3 *N* hydrochloric acid and dilute the solution to 500 ml. with 3 *N* hydrochloric acid. For ointments, transfer the sample to a separating funnel, dissolve in 50 ml. of ether, extract the solution with successive portions of 50 ml. of 3 *N* hydrochloric acid, collect the aqueous extracts, and dilute the mixture to 500 ml. with 3 *N* hydrochloric acid. Dilute an aliquot, containing 1 to 2 mg. of total sulphonamides, to 100 ml. with 3 *N* hydrochloric acid and determine the optical density of this solution at 239 and 280 mμ., using distilled water in the reference cell.

At the same time prepare solutions containing 1·5 mg. of sulphadiazine and 1·5 mg. of sulphathiazole in 100 ml. of 3 *N* hydrochloric acid and measure the optical density of these solutions and of 3 *N* hydrochloric acid as a correction blank. From the data obtained, compute the extinction coefficients and calculate the concentrations of sulphadiazine and sulphathiazole in the unknown solution from the equations

$$C_N = 1000\ (K_4E_{239} - K_2E_{280})/(K_1K_4 - K_2K_3)$$
$$C_L = 1000\ (K_1E_{280} - K_3E_{239})/(K_1K_4 - K_2K_3)$$

where K_1 = extinction coefficient for sulphadiazine at 239 mμ., K_2 = extinction coefficient for sulphathiazole at 239 mμ., K_3 = extinction coefficient for sulphadiazine at 280 mμ., K_4 = extinction coefficient for sulphathiazole at 280 mμ., C_N = concentration of sulphadiazine in unknown, C_L = concentration of sulphathiazole in unknown, E_{239} = optical density of unknown solution at 239 mμ., E_{280} = optical density of unknown solution at 280 mμ.

Values obtained for K_1, K_2, K_3, and K_4 (at 1·5 mg. per 100 ml. of 3 *N* acid) were 555, 106, 103, and 469, respectively. A. H. A. Abbott

Composition of Seed Fats of West Indian Citrus Fruits. H. C. Dunn, T. P. Hilditch, and J. P. Riley (*J. Soc. Chem. Ind.*, 1948, **67**, 199–203)—The dried seeds of West Indian grapefruits, oranges, and limes contain approximately

40 per cent. of fatty oils. Their constituent glycerides resemble those of cottonseed oil, except for the presence of small amounts of linolenic glycerides.

TABLE I

OIL CONTENTS OF CITRUS SEEDS AND CHARACTERISTICS OF THE OILS

	Grapefruit			
	"Foster"	"Marsh"	Sweet orange	Lime
(i) Seeds:				
Average wt. (50 seeds)	8·7 g.	8·0 g.	5·0 g.	3·0 g.
Shell, %	24	25	35·5	25
Kernel, %	76	75	64·5	75
Oil in whole seed, %	43	41	40	39
(ii) Oils:				
Saponification value	197·2	196·5	195·5	195·2
" equivalent	284·0	285·0	286·5	287·0
Iodine value	100·2	100·8	97·6	111·1
Free fatty acid (as oleic), %	5·7	4·6	22·5	3·3
Unsaponifiable matter, %	0·3	0·3	0·6	0·6
Refractive index at 25° C.	1·4687	1·4706	1·4686	1·4676

TABLE II

PROBABLE COMPONENT GLYCERIDES OF SWEET ORANGE, LIME, AND COTTONSEED OILS

	Sweet orange per cent. (mol.)	Lime per cent. (mol.)	Cottonseed per cent. (mol.)
Tri-saturated:			
Tripalmitin	—	2	—
Mono-unsaturated di-saturated:			
Dipalmito-oleins or -linoleins	19	10	8
Palmitostearo-oleins or -linoleins	8	15	5
Di-unsaturated mono-saturated:			
Palmito-oleo-linoleins	33	26	41
Palmito-dilinoleins	1	2	18
Palmito-linoleo-linolenins	6	20	—
Stearo-oleo-linoleins	10	4	—
Stearo-linoleo-linolenins	—	3	—
Tri-unsaturated:			
Dioleo-linoleins	1	—	—
Oleo-dilinoleins	15	8	28
Oleo-linoleo-linolenins	7	5	—
Dilinoleo-linolenins	—	5	—

The composition of the component acids, + unsaponifiable matter, of citrus seed oils was:—

TABLE III

	Grapefruit seed oil			
Acid (per cent.) wt.	"Foster"	"Marsh"	Sweet orange seed oil (neutralised)	Lime seed oil
Myristic	0·8	1·2	—	0·3
Palmitic	28·8	27·5	23·7	26·0
Stearic	2·1	2·9	8·3	9·5
As arachidic	0·6	2·1	0·7	0·5
Oleic	24·9	21·0	24·6	11·1
Linoleic	36·4	39·2	36·8	39·0
Linolenic	5·9	5·8	5·2	13·0
Unsaponifiable matter	0·5	0·3	0·7	0·6

E. B. DAW

Biochemical

N-Dimethyl-leucyl-glycine as a Buffer. J. Leonis (*C. r. Lab. Carlsberg Ser. Chim.*, 1948, 26, 357–360)—N-Dimethyl-leucyl-glycine is recommended as a chemically inert buffer for the physiologically important pH range between 7 and 8·5.

Procedure—Recrystallisation of N-dimethyl-leucyl-glycine—Suspend 20 g. of the powder in 100 ml. of chloroform and dissolve by dropwise addition of alcohol; shake after each addition. Add light petroleum (b.p. 40° to 60° C.) slowly until a faint turbidity is obtained and complete the crystallisation by storage for a few hours in a refrigerator. Filter, wash the residue with light petroleum, and dry *in vacuo* over phosphorus pentoxide. The recrystallised product has the formula

$$C_{10}H_{20}O_3N_2.1\tfrac{1}{2}H_2O.$$

Stock buffer solution—Dissolve 2·433 g. of N-dimethyl-leucyl-glycine and 1·159 g. of sodium chloride in water free from carbon dioxide and dilute to 50 ml.

Buffer solution A—Dilute 20 ml. of stock solution with 20 ml. of water free from carbon dioxide.

Buffer solution B—Mix 20 ml. of stock solution with 20 ml. of 0·2 *N* sodium hydroxide.

To prepare buffer solutions of known pH—Mix aliquots of buffer solutions A and B in accordance with Table I.

Table I

Mixture		pH (observed)	pK	pH (calc. from pK)
Soln. A ml.	Soln. B ml.			
0·50	4·50	8·77	7·82	8·76
1·00	4·00	8·42	7·82	8·41
1·50	3·50	8·20	7·83	8·17
1·75	3·25	8·09	7·83	8·07
2·00	3·00	7·98	7·80	7·98
2·25	2·75	7·89	7·81	7·89
2·50	2·50	7·80	7·80	7·80
2·75	2·25	7·70	7·79	7·72
3·00	2·00	7·61	7·79	7·63
3·25	1·75	7·53	7·80	7·54
3·50	1·50	7·41	7·78	7·43
4·00	1·00	7·19	7·79	7·20
4·50	0·50	6·79	7·75	6·85

The buffer does not react with carbonyl-containing compounds or carbon disulphide and, as it is not decomposed by ordinary peptidases, it can be used in proteolytic experiments.

The buffer has a relatively low temperature coefficient; Table II shows the values of pK over the temperature range 20° to 40° C.

Table II

T °C. ..	20°	22°	24°	26°	28°
pK (calc.)	7·86	7·82	7·79	7·76	7·72
T °C. ..	30°	32°	34°	37°	40°
pK (calc.)	7·69	7·66	7·63	7·58	7·53

A. H. A. Abbott

Method for the Quantitative Estimation of Theophylline in Blood and Urine: Application to the Dog. A. J. Plummer (*J. Pharmacol. Exp. Therap.*, 1948, **93**, 142–146)—The method described is based upon a reaction reported by Plummer and Mendenhall (*Ibid.*, 1938, **63**, 31) and is sensitive, technically simple, and applicable to both blood and urine, and the reagents are stable. Caffeine, theobromine, uric acid, ethylenediamine, sodium acetate, and normal blood constituents do not interfere.

Procedure—Add 5 ml. of a saturated solution of copper acetate in methyl alcohol to 4 ml. of a methyl alcohol solution of theophylline in a 15-ml. centrifuge tube and allow to stand in the tightly stoppered tube for 4 hr. to precipitate the theophylline copper compound quantitatively. Centrifuge for 15 min. at 1000 r.p.m. Decant the supernatant liquid, and drain, and wash the precipitate with 5 ml. of methyl alcohol. Centrifuge as before and again decant and drain. Dissolve the precipitate in 4 ml. of 0·2 *N* sulphuric acid and add 0·5 ml. of an aqueous solution containing 1 g. of potassium iodide per ml. Titrate the liberated iodine with 0·02 *N* sodium thiosulphate, using soluble starch as indicator. 1 mg. of theophylline is equivalent to 0·57 ml. of 0·02 *N* sodium thiosulphate.

Blood—De-proteinise by adding 25 parts of blood to 40 parts of 13 per cent. trichloroacetic acid solution. Allow to stand for 20 min.; filter or centrifuge. Make the solution just alkaline to litmus with 2·5 *N* sodium hydroxide and then add 10 ml. of a phosphate buffer (pH 8·0). The final pH must lie between 7·3 and 8·2. Extract the theophylline by shaking the buffered filtrate with three 20-ml. portions of a mixture of 3 volumes of chloroform and 1 volume of *iso*propyl alcohol for 5 min. in each extraction. Evaporate the combined extracts just to dryness on a water-bath and dissolve the residue in methyl alcohol by warming on the water-bath. Transfer the solution to a 15-ml. graduated centrifuge tube keeping the final volume of methyl alcohol between 0·3 and 0·5 ml., and evaporating if necessary. Complete the determination as previously described, using 0·005 *N* sodium thiosulphate, 2·28 ml. of which are equivalent to 1 mg. of theophylline.

Urine—Adjust the pH to between 7·3 and 8·2 by adding 2·5 *N* sodium hydroxide and one-fifth the volume of phosphate buffer of pH 8·0. Finish the estimation in a manner similar to that used for theophylline in blood. The volume of methyl alcohol used to dissolve the drug should be from 1 to 2 ml. Use 0·01 *N* sodium thiosulphate for the final titration; 1·14 ml. are equivalent to 1 mg. of theophylline.

Between concentrations of 6 and 100 mg. per cent. of theophylline in methyl alcohol, the volumes of thiosulphate used are directly proportional to the drug concentration. In dogs, an excretion of 1·8 per cent. in 90 min., after the injection of 8 mg. of theophylline per kg. of body weight, is reported. The method can be applied to human blood and urine.

G. A. Stewart

Agricultural

Colorimetric Method for the Estimation of 2 : 2-bis-(p-Chlorophenyl)-1 : 1 : 1-trichloroethane (DDT). F. R. Bradbury, D. J. Higgons, and J. P. Stoneman (*J. Soc. Chem. Ind.*, 1947, **66**, 65–68)—When *pp'*-DDT is warmed with a solution of hydroquinone in pure concentrated sulphuric acid a wine-red colour, characterised by strong absorption in the 480 to 500 mμ. band, is produced, and the reaction serves as a simple and rapid method for analysing commercial samples of DDT. The reagent is a 0·5 per cent. solution of pure re-crystallised hydroquinone in pure, concentrated, nitrogen-free sulphuric acid (*infra*). Purity of all the reagents used is essential.

Procedure—Heat an aliquot of the sample solution containing 0·2 to 1 mg. of DDT in a boiling-tube in a water-bath to remove the solvent, finally passing in a current of air to remove the last traces.

Remove the tube from the bath, add 0·5 ml. of pure, recently distilled diethyl sulphate, rotate the tube so as to dissolve all the DDT adhering to the sides, add 5·5 ml. of the hydroquinone reagent, and mix by shaking. Heat in boiling water for exactly 3 min. with occasional shaking and then cool the tube rapidly in running water. Make a blank determination, omitting the DDT. Compare the light absorption of the coloured solution and that of the blank in a Spekker absorptiometer, using No. 5 green filters and 1-cm. cells of capacity 6 ml. Within 20 min. of the formation of the colour ascertain the number of milligrams of DDT corresponding to the Spekker reading from a calibration curve. Under the conditions described, 0·1 mg. of DDT is just detectable.

The major impurities of commercial DDT, *viz.*, *op'*-DDT and *pp'*-DDD do not react sufficiently to interfere significantly with the method.

Since many DDT products are in coarse lumps and contain particles of extraneous matter, it is necessary to take large samples for analysis. The following method is recommended. Homogenise about 10 g. of the material by powdering in a mortar, dissolve 1 g. in 100 ml. of alcohol, and dilute 10 ml. of this solution to 500 ml. with alcohol. Take 5 ml. of this solution (*i.e.*, 1 mg. of the original material) for each determination. With amounts greater than 1 ml. an opalescence forms when the reaction tube is cooled after the colour development. This prevents accurate measurement of the light absorption in the absorptiometer.

The 98 per cent. sulphuric acid may contain enough nitrogen compounds to interfere seriously with the colour reaction by forming a brownish-red colour of lower intensity than the true colour given by the nitrogen-free reagent. To free analytical quality sulphuric acid from interfering nitrogen compounds, heat 1 litre with 2 g. of ammonium carbonate at 150° C. for 4 hr. A. O. Jones

Rapid Conductometric Method for Estimating Gypsum in Soils. C. A. Bower and R. B. Huss (*Soil Science*, 1948, **66**, 199–204)—The gypsum is extracted from the soil with water and then precipitated by adding acetone. The solid is re-dissolved in water and the calcium sulphate content determined from a measurement of the conductivity of the solution.

Procedure—Weigh 10 to 20 g. of air-dried soil into a bottle, add sufficient water to dissolve all the gypsum present, stopper the bottle, and shake the mixture mechanically for 30 min. (To avoid conversion of the gypsum, $CaSO_4.2H_2O$ into $CaSO_4.0{\cdot}5H_2O$, the soil should not be over-heated.) After filtering off the undissolved material, transfer 20 ml. of the extract, containing 0·1 to 0·6 mg. equivalents of calcium sulphate, to a centrifuge tube, add 20 ml. of acetone, and allow the precipitate to flocculate. Centrifuge the mixture, wash the solid residue with 10 ml. of acetone, and again centrifuge the solution. After draining off all the acetone, add 40 ml. of water to dissolve the solid, and measure the conductivity of the resulting solution. Calculate calcium sulphate content from a calibration curve constructed from the following figures:

Mg.-equivalents of calcium sulphate per litre	Electrical conductivity in milli-ohms per cm. at 25° C.
1	0·121
2	0·226
5	0·500
10	0·900
20	1·584
30·5	2·205

The results obtained by this method are in good agreement with those obtained by the usual method involving the separate determination of calcium and sulphate. None of the common ions found in soil interferes with the determination. J. G. Waller

Organic

Determination of Glycerol in Fermentation Residues. P. J. Elving, B. Warshowsky, E. Shoemaker, and J. Margolit (*Anal. Chem.*, 1948, **20**, 25–29)—In complex solutions such as fermentation residues glycerol is determined by measuring the formaldehyde produced on oxidation by periodic acid. Substances that interfere by themselves forming formaldehyde with periodic acid are removed by treatment with an alcoholic solution of lime. The formaldehyde can be determined by any convenient standard method; the polarographic method and the sulphite method with electrometric titration are the most suitable for routine work.

Procedure—Weigh sufficient of the sample to contain approximately 150 mg. of glycerol, dilute to 50 ml. with water, transfer 10 ml. of the solution to a small conical flask containing 1 g. of lime, and heat at 50° C. on a water-bath for 30 min. Add 50 ml. of 95 per cent. ethyl alcohol, stir, and filter by decantation through a Buchner funnel, using gentle suction and a fine filter paper. Wash the residue five times with 5-ml. portions of 95 per cent. ethyl alcohol saturated with lime. Mix the filtrate and washings, add 20 ml. of water and 1 ml. of 30 per cent. sodium hydroxide solution. Evaporate until about 20 ml. of solution remain, neutralise to methyl red with 2 N sulphuric acid, transfer the mixture to a Kjeldahl flask, and dilute to approximately 50 ml. with water.

Add 5 ml. of 0·5 N periodic acid and a few glass beads. Attach the Kjeldahl flask to a spray-trap and thence to a vertical, water-cooled condenser, the end of which is covered to a depth of 2 cm. by 50 ml. of water contained in a beaker. Heat the contents of the flask gently at first and then distil the solution at the rate of 3 to 4 ml. per min. until about 5 ml. remain in the flask. Near the end of the distillation lower the receiver so that the end of the condenser is free from the surface of the distillate.

Determine the formaldehyde in the distillate by any convenient method. A. H. A. Abbott

Potentiometric Titration of Weak Acids in Anhydrous Ethylenediamine. M. L. Moss, J. H. Elliott, and R. T. Hall (*Anal. Chem.*, 1948,

20, 784–788)—Phenols and other acids that are too weak to be titrated in aqueous solution give sharp end-points if the titration is carried out with anhydrous ethylenediamine, or a similar suitable basic material, as solvent. The titration is carried out with a solution of sodium aminoethoxide in anhydrous ethylenediamine as the base, and the end-point is detected by means of a pair of antimony electrodes.

The apparatus used excludes all atmospheric moisture and carbon dioxide, and the tip of the burette reaches below the surface of the liquid to be titrated. One antimony electrode is immersed in the solution, which is stirred by means of a magnetic stirrer. The second antimony electrode is mounted in the burette below the stopcock.

Reagents—The anhydrous ethylenediamine is prepared by drying the commercial material over sodium hydroxide and distilling it over sodium. To prepare the solution of sodium aminoethoxide, dissolve 2·5 g. of sodium in 100 ml. of anhydrous ethanolamine, which has been three times fractionated, and dilute the solution to 500 ml. with ethylenediamine. The sodium should first be washed with ethyl alcohol and ethanolamine.

Procedure—Dissolve 0·1 to 1·0 g. of material in 75 ml. of ethylenediamine and titrate the solution with the sodium aminoethoxide solution. The titrant solution is standardised by titrating a weighed sample of benzoic acid in the same way.

The method has been used for the determination of weakly acidic groupings in resins and for the titration of amino acids. J. G. Waller

Constitution and Analytical Applications of Methylene Blue Periodide. M. J. A. Gautier (*Bull. Soc. Chim.*, 1948, 836–838 M)—Different formulae have been attributed to the compound that precipitates when solutions of methylene blue and iodine are mixed. These formulae were based upon experiments on the volumetric applications of the reaction. The constitution of the compound has now been established by its isolation and analysis. The periodide was precipitated from a solution of 0·5 g. of methylene blue in 500 ml. of water by addition of 100 ml. of 0·1 *N* iodine. The washed and dried precipitate is a brown powder melting with decomposition at 126° to 127° C. (block). Its iodine content (determined by reduction with zinc and sodium hydroxide and precipitation as silver iodide) corresponds to the formula $C_{16}H_{18}N_3S.HI.2I_2$. Confirmation is provided by the fact that dilute solutions of methylene blue and iodine decolorise one another when mixed in proportions corresponding to this formula.

The periodide yields practically no iodine to water or to solutions of the alkali iodides, but is decomposed by a number of other reagents such as sodium thiosulphate, alkaline reducing agents, and unsaturated compounds, with regeneration of the blue colour of the dye. Aqueous solutions also turn blue in contact with many organic solvents such as chloroform, ether, carbon disulphide, alcohols, and pyridine.

Detection of stannous ions—Using a plate, add 1 drop of 0·01 *N* iodine to 1 drop of 0·01 per cent. methylene blue solution. To the colourless mixture add a drop of a dilute solution of a stannous salt. An immediate blue colour results: sensitivity 1 μg.; dilution limit 10^{-5}. The reaction can also be carried out on filter paper. A large excess of stannous ions discharges the colour by reducing the dye to the leuco base.

Detection of mercuric ions—The procedure is the same as for stannous ions, but an excess of mercuric salt does not discharge the blue colour.

Use as an indicator in iodimetry—This application was recommended by Sturdy and Sinnatt (*Analyst*, 1910, 35, 309). An aqueous suspension of the periodide or a drop of 0·1 per cent. methylene blue solution is added to the iodine solution. On titration with thiosulphate a change to blue occurs at the equivalence point.

The compound also behaves as an indicator in acidimetry, but has no advantage over the indicators commonly employed. W. C. Johnson

Determination of Substances Containing an Alkyl Pyridinium Group. E. Kahane and O. Sackur (*Bull. Soc. Chim.*, 1947, 239–242 M)—On oxidation of *N*-alkyl pyridinium compounds in alkaline solution the following have been observed: (1) formation of an α-pyridone, (2) migration of the alkyl group to the α- or γ-position, and (3) rupture of the nucleus with formation of glutaconic aldehyde and an alkyl amine. Conditions under which most compounds of this type react quantitatively according to (3) have been devised.

Procedure—The determination is best carried out on a micro-analytical scale. Take 5 to 25 mg. of the substance for analysis, 5 to 20 ml. of concentrated sodium hydroxide solution, and 5 ml. of a saturated solution of potassium permanganate, and distil from a Parnas-Wagner apparatus, titrating the distillate progressively with 0·02 *N* sulphuric acid until no more base distils. The time required is generally 10 to 20 min. A mixed indicator of methylene blue and methyl red is suitable.

The limits of error were $-1{\cdot}7$ and $+1{\cdot}6$ per cent. when the method was applied to the following substances: methyl pyridinium iodide, ethyl pyridinium bromide, hydroxyethyl pyridinium chloride, nicotinic acid methiodide, methyl nicotinate methiodide, nicotinamide methiodide, picolinic acid methiodide, α-picoline methiodide, and γ-picoline methiodide.

The probable general course of the decomposition is discussed and the distilled bases are shown, by analysis of their phosphotungstates, to be variable mixtures of alkylamines and ammonia.

W. C. Johnson

Colorimetric Method for the Determination of Alginic Acid in Seaweed Specimens. E. G. V. Percival and A. G. Ross (*J. Soc. Chem. Ind.*, 1948, **67,** 420–421)—Seaweed (0·1 g.) of known moisture content is left standing overnight, in a 30-ml. beaker with 10 ml. of 0·2 *N* sulphuric acid, filtered through a 1-inch funnel and washed with water and the filter paper transferred to a 100-ml. beaker containing 20 ml. of 3 per cent. sodium carbonate solution. The paper is disintegrated by means of two glass rods. After the solution has been kept at 50° C. for 2 hr., it is stirred occasionally at room temperature and finally kept overnight, then filtered through a 9-cm. fluted filter into a 100-ml. flask, washed with warm water, and diluted to the mark. Three ml. are put in a test tube (8 in. × 1 in.) fitted with a high-speed stirrer, driven by a "Quickfit" chuck. The bottom end of this tubular stirrer is perforated for about 1·5 in. with a small hole through which compressed air is blown during stirring. To the tube, in a bath of ice and salt, 18 ml. of concentrated sulphuric acid (A.R.) are added from a burette—approximately 9 ml. at a rate of 1 drop per sec. and the rest at a faster rate.

After addition of the acid, the tube is kept for exactly 20 min. in vigorously boiling water, after which half the contents are used as a blank, and the rest is kept in a bath at 80° C. for 5 min. and 0·3 ml. of a 0·2 per cent. carbazole solution in ethanol is then added. (The carbazole is re-crystallised twice from glacial acetic acid.) After 45 min., the blank and sample are compared in a Spekker absorptionmeter, model H560, a green filter being used. The percentage of alginic acid is found from a calibration curve. Quantitative details for micro-determinations are also given.

E. B. Daw

Anthrone for Estimating Low Concentrations of Sucrose. E. E. Morse (*Anal. Chem.*, **1947, 19, 1012–1013**)—The Molisch α-naphthol test for sucrose, being a ring test, cannot be adapted readily to transmittancy measurements. Dreywood (*Ind. Eng. Chem., Anal. Ed.*, 1946, **8**, 499; *Analyst*, 1946, **71**, 544) suggested anthrone dissolved in concentrated sulphuric acid as a qualitative reagent for carbohydrates, and the extreme sensitivity of this test and the fact that it is not a ring test suggested its development for the quantitative determination of sucrose in low concentrations such as 50 to 250 p.p.m., *e.g.*, in the condensed vapours and solutions of sugar processing factories.

Procedure—To 2 ml. of the solution to be tested contained in a clean, dry Pyrex test tube (15 × 125 mm.) add 3 ml. of a 0·05 per cent. solution of anthrone in concentrated sulphuric acid (prepared afresh every 3 or 4 days) so as to form a lower layer, and then shake the tube to effect complete mixing. Measure the transmittancy of the solution for white light in a photo-electric colorimeter against distilled water as standard with a transmittancy of 1·00. By reference to a previously determined calibration curve or table, ascertain the concentration of sucrose in the original sample.

In mixing the sample and reagent, the tube should be shaken as one shakes a clinical thermometer. Alternatively, a small glass stirrer can be used. Since the reaction is highly sensitive to temperature, it is important that the test be performed in a standardised manner. Variations in the procedure will alter the temperature rise caused by the heat of dilution of the acid, and hence change the amount of blue-green colour produced by a given concentration of sucrose.

The anthrone used was prepared by the method in *Organic Syntheses* (Collective Vol. I, p. 60). Since the presence of an impurity was suspected, the anthrone was re-crystallised from glacial acetic acid and yielded material, the colour of which slowly changed to orange-green on long standing. A second re-crystallisation from the same solvent was of benefit, but not a third. A. O. Jones

Colour Test for Identification of Glucose. S. Hestrin and J. Mager (*Anal. Chem.*, 1947, **19, 1032–1035**)—The test is based on the fact that glucose, unlike many other sugars examined, including the aldohexoses, galactose, and mannose, forms no colour when heated with phosphoric acid alone under the conditions described but forms a characteristic lilac colour when pyrocatechol is subsequently added to the reaction system.

Procedure—To an amount of the powdered sample containing 0·2 to 1·0 mg. of carbohydrate in a dry test tube, preferably with a ground-glass stopper, add 1 ml. of 85 per cent. orthophosphoric acid. (If the sample is in solution add 0·05 ml. of the sugar solution to 1 ml. of 90 per cent. phosphoric acid.) Heat the tube in boiling water for 15 min., and shake after 1 min. to hasten dissolution. If no colour forms, heat for a further 15 min. If still no colour forms, add to the warmed solution 4 ml. of a freshly prepared 0·2 per cent. solution of pyrocatechol in 85 per cent. phosphoric acid, heat the mixture for 15 min., and compare the colour with that of a control made simultaneously with glucose.

Group (*a*)—Glucose, glucosamine, diglucoses (maltose, trehalose), polyglucoses (starch, glycogen, cellulose), and glucuronic acid form no colour when heated for 15 min. with phosphoric acid alone.

Group (*b*)—Mannose, galactose, pentoses (xylose, arabinose, lyxose, ribose), methyl pentose (rhamnose), ketoses (sorbose, fructose), oligosaccharides containing a non-glucose unit (sucrose, melibose, raffinose), and polysaccharides containing a non-glucose unit (inulin, mannan) form a greenish-yellow to amber-brown colour when heated for 15 min. in phosphoric acid. (Galactose, lactose, and melibose give a relatively weak colour. If galactose is present in very small amount, the weak amber colour may be difficult to see. However, the red colour formed with pyrocatechol distinguishes galactose from glucose.)

Absence of colour after heating with phosphoric acid alone shows that none of the sugars in group (*b*) is present. If the amount of carbohydrate added is large enough (more than 2 mg. per ml.), the different glucoses (glucose, maltose, glycogen, starch, and cellulose) yield a pink colour when heated with phosphoric acid for 30 min.

The relative amounts of colour formed with phosphoric acid by these common aldohexoses were plotted against the sugar concentration. The extinction - concentration curves were all straight lines and there was a particularly large difference between glucose and mannose in colour-forming activity. In a mixture of equal parts of glucose and mannose the amount of colour produced is within 3 per cent. of the colour produced by the mannose constituent alone. A study of the application of this reaction to the determination of mannose in presence of glucose seems desirable.

Differentiation of glucose, galactose, and mannose by the reaction with pyrocatechol was studied in greater detail. Transmission was measured by means of a Pulfrich Step-photometer, usually with a solution layer of 1 cm. A characteristic feature of the glucose product is its marked absorption peak in the region of 500 mμ. (filter S50). The ratio $\epsilon_{S43}/\epsilon_{S50}$ is different enough to provide a clear-cut distinction between these three substances. The ratio, though sensitive to differences of configuration within the sugar unit, is independent of the nature of the interglucosidic linkage, the polymerisation degree of the sugar unit, and the carbohydrate concentration. Although it was difficult to obtain good reproduction of the absolute amount of colour formed by a given sugar, the ratio $\epsilon_{S43}/\epsilon_{S50}$ remained constant to within ±7 per cent.

The method is valid as a means of identifying glucose only if no other colour-forming substance is present in the test material. Common pentoses (D- and L-arabinose, ribose, xylose, lyxose, and rhamnose) when heated in the pyrocatechol reagent produce colours that are intermediate in tone between the red-brown given by galactose and the lilac given by glucose. Moreover, two ketoses (fructose and sorbose) and one pentose (xylose) yield colours that are not certainly distinguishable in terms of $\epsilon_{S43}/\epsilon_{S50}$ from the colour given by glucose. It is particularly fortunate that the presence of non-glucose sugars (hexoses and pentoses) is revealed by their colour in the first step of the standard procedure, *i.e.*, on heating with phosphoric acid alone, and specific testing for these interfering sugars is therefore unnecessary. Glucuronic acid, on heating with pyrocatechol, forms a red-brown colour different from that given by glucose. Like glucose, however, it forms no colour when heated with phosphoric acid alone. It is desirable therefore to demonstrate the absence of glucuronic acid by independent means.

Identification of glucose by this test is necessarily presumptive. It is always possible that units different from glucose but simulating glucose in the test can occur in biological material.

Tryptophan and nitrites interfere with the test. The absence of interfering colour formation in the presence of any amino acid, with the exception of tryptophan, is an important merit of the method. As may be expected, tryptophan-free proteins such as gelatin, do not interfere. The reaction can be applied safely in presence of certain proteins containing tryptophan, *e.g.*, edestin, yet, clearly, caution is necessary where the test is being applied to an unknown protein material containing tryptophan. Plasma albumin and β-lactoglobulin interfere with the test. A. O. JONES

Inorganic

Accuracy of Estimation of Hydrogen Peroxide with Potassium Permanganate Titration. C. E. Huckaba and F. G. Keyes (*J. Amer. Chem. Soc.*, 1948, **70**, 1640–1644)—The trustworthiness of this method has been questioned. Decomposition of hydrogen peroxide by a catalyst and measurement of the volume of oxygen evolved is chosen as the only trustworthy method with which the titration methods could be compared. Results of analyses by the volumeter method are compared with those obtained with potassium permanganate titration under various conditions.

The decomposition method is too inconvenient for general use. The sample of 2 to 3 per cent. hydrogen peroxide was weighed in a tube which was then attached to the cooled decomposition vessel. The liquid was run into the vessel, which was cooled in "dry ice" and the tube was rinsed with water. The rinsing water was run into the decomposition vessel under vacuum. Osmic acid solution or lead oxide suspension as catalyst was added in a similar manner to the frozen hydrogen peroxide solution. The gas evolved as the liquid warmed was pumped into a volumeter by a Toepler pump.

The glass apparatus used in the potassium permanganate method was made from glass previously treated with hot fuming sulphuric acid. A 10-g. sample of 2 to 3 per cent. hydrogen peroxide was weighed in a glass-stoppered flask and rinsed with about 50 ml. of conductivity water into a beaker containing 150 ml. of conductivity water and 7 ml. of 95 per cent. sulphuric acid. Approximately 0·16 *N* potassium permanganate, standardised by the method of Fowler and Bright (*J. Res. Nat. Bur. Standards*, 1935, **15**, 493), was run in from a 500-ml. weight burette at 30 to 40 ml. per min. with moderate stirring.

The deviation between the results obtained by the two methods for the same sample on the same day was with one exception not greater than 1 in 300. The average deviation for nine comparisons was 1 in 2500. The comparison between the two methods was equally favourable with osmic acid and with lead oxide as catalyst. The results obtained by the permanganate method were still concordant with those by the decomposition method when double the acid concentration was used, and when the titration rate was 10 ml. per min., and 50 ml. per min. To avoid the formation of manganese dioxide it is advisable to perform the titration at a rate of less than 40 ml. per min. and with a ratio of sulphuric acid to hydrogen peroxide of at least 60 to 1 by weight.

B. ATKINSON

Action of Mercury on Various Compounds. E. Montignie (*Bull. Soc. Chim.*, 1947, M 377–378)—The interaction of mercury with solutions of various oxidising agents is described as follows. Each experiment is conducted at room temperature with continuous agitation. *Sodium perborate*—Oxygen evolution begins after 1 hr. and continues until the perborate is completely decomposed. The mercury is not oxidised. *Potassium permanganate*—The products obtained with a 0·5 *N* solution are hydrated manganese dioxide, potassium manganate, and mercurous oxide. *Ammoniacal silver oxide*—Silver is deposited and the mercury is partially oxidised to mercurous oxide. *Sodium nitrite*—In 5 hr., a small amount of ammonia is formed and some mercurous oxide. *Sodium arsenate*—Sodium arsenite and mercurous oxide are formed. *Potassium ferricyanide*—A greenish-blue precipitate, consisting of Turnbull's Blue and mercuric oxide, is slowly produced. *Sodium persulphate*—The products obtained after 6 hr. are sodium sulphate, sulphuric acid, and basic mercuric sulphate. *Potassium cyanate*—Soluble mercuric oxycyanide and potassium hydroxide are formed (3 hr.). *Potassium percarbonate*—Oxygen is evolved and the mercury remains unchanged.

W. C. Johnson

Automatic Potentiometric Titration of Iron and Titanium with Chromous Ion. J. J. Lingane (*Anal. Chem.*, 1948, **20**, 797–801)—The conditions for the potentiometric titration of titanic and ferric ions with chromous ions have been investigated with an automatic titration apparatus (*cf. Ibid.*, 1948, **20**, 285; *Analyst*, 1949, **74**, 219). The same conditions apply to electrometric titrations carried out with manual apparatus.

Titration of ferrous ions—A platinum indicator electrode and a saturated calomel reference electrode are used, and the titration is carried out in solutions that are 1·8 *M* with repsect to sulphuric acid.

Titration of titanic ions—In this case a mercury indicator electrode is used in conjunction with a saturated calomel reference electrode, and the solution should be approximately 4·0 *N* with respect to sulphuric acid. Chloride solutions are less suitable.

Titration of mixtures of ferric and titanic ions—The standard potentials of the ferrous-ferric and titanous-titanic couples are sufficiently separated for $Fe^{\cdot\cdot\cdot}$ and $Ti^{\cdot\cdot\cdot\cdot}$ to be titrated in the same solution. The solution is made 4·0 *N* with respect to sulphuric acid, and a platinum reference electrode is used until the ferrous-ferric end-point has been slightly passed. The mercury electrode is used for completing the titration.

In addition, small amounts of iron can be determined in presence of large amounts of titanium. For the determination of small amounts of titanium in presence of excess of iron, the ferric ions are first reduced to the ferrous state with sulphur dioxide, and after boiling off the excess of sulphur dioxide, the titanium is titrated.

J. G. Waller

Separation of Nickel and Cobalt in the Micro-Estimation of Copper with Diethyldithiocarbamate. H. Cheftel, J. Bail, R. Fouasson, and P. Clavié (*Bull. Soc. Chim.*, 1947, M 311–313)—The authors are concerned with estimating traces of copper in fresh vegetables and in canned vegetables and in avoiding interference from metals derived from machinery and from the packages. The metals considered are iron, tin, nickel, chromium, manganese, and cobalt. Addition of sodium pyrophosphate in sufficient quantity prevents interference by iron (MacFarlane, *Biochem. J.*, 1932, **26**, 1022) and by manganese. Neither tin nor chromium interferes with the determination of 10 to 50 μg. of copper when present in 100-fold excess. Nickel yields a yellow colour with diethyldithiocarbamate and interferes when present in 3-fold excess; it is removed by precipitation with dimethylglyoxime and extraction of the nickel complex with chloroform. Cobalt gives a green colour with the copper reagent but this effect is also eliminated by the addition of dimethylglyoxime, although the cobalt - glyoxime compound is not extracted by the chloroform.

Procedure—Destroy the organic matter by heating with mixed nitric, sulphuric, and perchloric acids, evaporate until white fumes appear, cool, dilute, and neutralise to litmus paper with concentrated aqueous ammonia solution. Transfer to a volumetric flask and dilute to the mark. The solution at this stage should contain 1 to 10 mg. of copper in 1 litre. Test 2 to 3 ml. of this solution for nickel by adding concentrated aqueous ammonia solution until the pH is at least 9·0, followed by 2 to 4 drops of a 1 per cent. solution of dimethylglyoxime in 95 per cent. alcohol, and heating in a water-bath at 80° C. for 10 min. If no rose colour appears proceed directly to the estimation of copper. If a positive reaction for nickel is obtained proceed as follows. Take an aliquot containing 10 to 25 μg. of copper, add 5 to 10 ml. of a 6 per cent. w/v solution of tetrasodium pyrophosphate, the greater quantity if the solution contains as much as 500 mg. of iron plus manganese, per litre. Heat in a water-bath at 80° C. for 15 min. To the hot solution add an excess of dimethylglyoxime solution and concentrated aqueous ammonia dropwise until the nickel is precipitated. A large excess of ammonia renders difficult the subsequent extraction of the precipitate. Cool to 50 to 55° C. and shake for 1 to 2 min. in a glass-stoppered tube with 2 ml. of chloroform. Transfer to a separating funnel, rinsing the tube with two 2-ml. portions of chloroform and then twice with 1 to 2 ml. of water. Separate the chloroform layer and wash the aqueous layer with two 2-ml. portions of chloroform. Wash the mixed chloroform extracts with two 3-ml. quantities of water and add the washings to the original aqueous layer. To the aqueous liquid, contained in a separating funnel, add 0·5 ml. of 2 per cent. sodium diethyldithiocarbamate solution and 5 ml. of *iso*amyl alcohol, shake for 1 min., separate and pass the alcoholic layer through a dry filter. Compare the colour of the filtrate with a suitable series of standards similarly prepared or, preferably, use a photo-electric absorptiometer calibrated for the method.

The procedure permits the estimation of quantities of copper of the order of 25 μg. to within ±1 μg.

W. C. Johnson

Estimation of Quartz in Presence of Silicates. S. S. Gurvits and V. V. Podgayts (*Zavod. Lab.*, 1948, **14**, 935–938)—The determination of quartz in siliceous dust is important for assessing silicosis risk. The Soviet specification GOST 1324–47 limits the concentration of dust containing 50 per cent. of quartz to 2 mg. per cu. metre, but permits 10 mg. per cu. metre of other siliceous dusts.

Tests were carried out on various silicate powders containing 95 per cent. of particles, of diameters up to 2 mμ., using hydrofluoboric and hydrofluosilicic acids as selective solvents of non-quartz silica.

Tests with hydrofluoboric acid—To 0·2 g. of the material in a platinum crucible were added 5 ml. of hydrofluoboric acid solution (approximately 27 per cent.) and, to remove the effect of the fluoride ion, 2 ml. of 2 per cent. ferric chloride solution. The insoluble matter was filtered off, washed twice with 5 ml. of *N* hydrochloric acid and three times with 5 ml. of hot water, and ignited to constant weight. Tests were carried out at room temperature and at 45° to 50° C. The duration of the reaction ranged from 24 hr. to 5 days.

Quartz was practically unchanged after 5 days. Dissolution of the silicates took place but incompletely. At 45° C., 67 to 70 per cent. of the kaolin and hornblende, 50 to 60 per cent. of the felspar and talc, and 80 to 90 per cent. of the biotite and serpentine were dissolved.

Tests with hydrofluosilicic acid—To 0·2 g. of the powder moistened with water, were added 5 ml. of hydrofluosilicic acid and the undissolved matter was collected, washed, and ignited after 1 to 10 days. After 4 to 5 days the residues of the silicates were between 1 and 10 per cent. but in most cases contained no silica. In the first day quartz lost 2·5 to 3 per cent. and, at the end of 10 days, 11 per cent. Further experiments showed that the dissolution of the silicates was hastened by preliminary treatment with hydrochloric or sulphuric acid.

Procedure—Place 0·1 to 0·2 g. of the finely ground material (particle size 2 to 3 mμ.) in a platinum crucible, add 5 to 10 ml. of concentrated hydrochloric acid, heat carefully on a sand-bath for 10 to 15 min., add 5 ml. of water, boil, filter through a close paper, wash twice with 5-ml. portions of hot, diluted hydrochloric acid (1 + 5) and three times with 5-ml. portions of hot water, and return the filter and insoluble matter to the platinum crucible. Ash the paper at a low heat and then ignite for 20 to 30 min. at a white heat. Cool, add 5 ml. of 48 per cent. hydrofluosilicic acid, cover with a platinum lid, and leave at room temperature for 24 to 30 hr. Filter through a close paper, wash three times with 5-ml. portions of hot water, ash, ignite to constant weight at white heat, treat with sulphuric acid and hydrofluoric acid, evaporate, ignite, and weigh again. The difference in weight gives the quartz content.

With equal parts of quartz, biotite, talc, and kaolin and with equal parts of quartz, felspar, serpentine, and garnet, theoretical results were obtained. Quartz was under-estimated by about 5 parts in 100 in presence of serpentine only, and over-estimated by a few parts in 100 in presence of felspar only, kaolin only, or hornblende only.

G. S. SMITH

Colorimetry of Aluminium Oxinate. M. M. Raynes and Yu. A. Larionov (*Zavod. Lab.*, 1948, **14**, 1000).—For extracting the aluminium salt of 8-hydroxyquinoline from aqueous solutions, Kuskova (*J. Anal. Chem. Russ.*, 1947, **2**, 7; *Analyst*, 1948, **73**, 114) recommended *iso*amyl alcohol and rejected benzene, and Mervel (*J. Anal. Chem. Russ.*, 1947, **2**, 103; *Analyst*, 1948, **73**, 172) recommended benzene, finding *iso*amyl alcohol to be unsuitable. Gentry and Sherrington (*Analyst*, 1946, **71**, 432) used chloroform.

Mervel's objections to *iso*amyl alcohol have now been confirmed. Tests were carried out with the solvents mentioned above and also with carbon tetrachloride, light petroleum, and butyl acetate. The limits of visibility of the colour, in mg. of aluminium per 10 ml., are 0·002 for carbon tetrachloride, chloroform, and benzene, 0·005 for butyl acetate, 0·01 for light petroleum, and 0·02 for *iso*amyl alcohol. Carbon tetrachloride has certain advantages over the other solvents: colours are better defined and the solutions are more transparent.

G. S. SMITH

Rapid Electrometric Method of Determining Aluminium in Ores. S. K. Chirkov (*Zavod. Lab.*, 1948, **14**, 783–787)—Well-polished aluminium and nichrome wires are suitable as electrodes in the non-compensated electrometric titration of aluminium with sodium fluoride in acetic acid solutions.

The quantitative relation between aluminium and fluoride ions, as indicated by the titration curves for dilute aluminium solutions ($\ngtr$ 0·005 *M*) from the points of intersection of tangents, depends on the pH. With pH 3·5 to 4·5, the ratio Al : F at the equivalence point is 1 : 3, with pH 6·0 it is 1 : 6, with pH $<$ 3·5 it becomes $>$ 1 : 3, but with pH $<$ 3 no interaction occurs, *i.e.*, the galvanometer reading rises steadily from the beginning of the addition of fluoride and then flattens out, whilst in the less acid solutions the reading is almost constant until the equivalence point is approached, and then it rises rapidly.

With more concentrated aluminium solutions titrated with fluoride solutions of concentration greater than 0·2 *M*, *e.g.*, 0·01 *M* aluminium chloride titrated with 0·5 *M* sodium fluoride, there are two equivalence points, the complete curve consisting of an initial horizontal portion, a rise to a maximum, followed by a fall, and then a second rise followed by a flattening-off. The intersections of the tangents to the rising portions with the tangent to the initial part of the curve correspond to the ratios Al : F of 1 : 3 and 1 : 6. This effect is shown only when the aluminium concentration exceeds the solubility of the cryolite that is formed. Addition of about 2 to 3 g. of sodium chloride per 50 ml. eliminates the maximum and the minimum, and only one equivalence point, corresponding to the ratio 1 : 6, is obtained. This point is shown by a definite break in the curve and hence it can be found, without the drawing of a curve, merely by observation of the galvanometer.

Apparatus—Wires of aluminium and nichrome, 3 mm. thick and 16 cm. long, are carefully polished with fine glass-paper and filter paper, and the lower ends are filed round. The cleaning and polishing must be carried out before each titration. The upper ends of the electrodes are securely clamped to flexible well-insulated copper wires. A small glass stirrer is joined to the electrode pair, which should be separated as far as the beaker will allow. They are immersed to a depth of 1 to 1·5 cm. in the solution to be titrated. The electrodes are connected in series with a 20,000- to 30,000-ohm fixed resistance and a pointer galvanometer with a 17-mv. scale having 0·1- or 0·2-mv. divisions. Stirring is carried out by rotation of the platform on which the beaker rests.

Sodium fluoride solution—Prepare 0·3 to 0·4 *M* sodium fluoride, neutralise the excess of alkali with hydrochloric acid, using methyl orange, and keep the solution in a paraffined bottle. Standardise on approximately 0·05 *M* aluminium potassium sulphate, the aluminium content of which has been determined gravimetrically or by other means. To 10 ml. of the aluminium solution add 2 ml. of diluted hydrochloric acid (1 + 1), 10 ml. of 20 per cent. sodium chloride solution, and 10 ml. of 20 per cent. sodium acetate solution, dilute to 45 to 50 ml., make just alkaline to methyl orange with 8 to 10 per cent. sodium hydroxide solution, and titrate electrometrically with the sodium fluoride solution, obtaining the full titration curve.

Procedure—Fuse 3 g. of sodium hydroxide in an iron crucible, add 0·2 to 0·5 g. of the ore to the cooled melt, fuse again, cool, extract with water, warming slightly, transfer the solution to a 200-ml. graduated flask, cool, make up to the mark, allow the insoluble matter to settle, and filter through a dry filter, rejecting the first 10 to 20 ml. of the filtrate. Transfer 100 ml. to a beaker, neutralise to methyl orange with diluted hydrochloric acid (1 + 1), and add an excess of 2 to 3 ml. of this acid. Evaporate to 25 to 30 ml., cool, add 10 ml. of 20 per cent. sodium acetate solution or 2 g. of the pure salt, neutralise to methyl orange, and titrate with the sodium fluoride solution.

If a maximum appears or if the galvanometer needle becomes erratic as the equivalence point is approached, repeat the titration after adding 5 to 10 ml. of 20 per cent. sodium chloride solution.

Bauxites with aluminium oxide contents of from 17·5 to 41·4 per cent., and iron, and titanium ores containing about 5 per cent. of aluminium oxide gave results correct to within 1 or 2 parts in 100.

G. S. SMITH

Physical Methods, Apparatus, etc.

Preparation of Aluminium Oxide for Chromatography. G. Dupont, R. Dulou, and M. Vilkas (*Bull. Soc. Chim.*, 1948, 785–786 M)—Aluminium hydroxide of commerce is used as the starting material in a study of the conditions for ignition and activation. At 200° to 350° C., a monohydrate of low activity is obtained; at 350° to 450° C., a highly absorbent amorphous powder results, suitable for use as a desiccant but too fine for chromatography. At 450° to 500° C., the oxide changes to the granular, crystalline γ-form which, after further treatment, has the desired characteristics. The α-modification, a non-absorbent variety, begins to form at 800° C.

Procedure—Heat aluminium hydroxide for 1 hr. at 500° C. Cool, and mix the oxide with sufficient concentrated hydrochloric acid to cover it. Allow to stand for 2 to 3 hr., with occasional stirring. Then stir with a large volume of water and decant after 5 min. so as to remove the finer powder still in suspension. Repeat the treatment with water until the oxide settles completely in 2 to 3 min. (5 to 6 treatments). Filter off the product, wash it free from acid, and place it in an oven at 105° C. Control the drying by measurement of the absorptive power according to the standardised procedure of Brockmann (Ber., 1941, **74**, 73) and store the product in a bottle with a ground-glass stopper.

To fill a chromatographic column the following method is recommended. Close the bottom end of the column and fill it two-thirds full with an appropriate solvent. Add the dry aluminium oxide in a thin stream from a funnel with a constricted stem, revolving the column slowly on its own axis during the process. W. C. JOHNSON

Removal of Metals at Mercury Cathode. Separation of Interfering Metals in the Determination of Aluminium, Alkaline Earth and Alkali Metals. T. D. Parks, H. O. Johnson, and L. Lykken (*Anal. Chem.*, 1948, **20**, 148–151)—Operating conditions for the self-contained electrolysis cell previously described (Johnson *et al.*, *Ibid.*, 1947, **19**, 481; *Analyst*, 1948, **73**, 476) have been investigated.

Operating conditions—Observations were made of the effect of current and distance between electrodes on the efficiency of removal of metals from solution. The most complete deposition after electrolysis for 30 min. is obtained by using a current of 5 amp. from the 5-volt supply and a distance between the electrodes of 8 to 10 mm. The efficiency of deposition in a fixed time rises with an increase in area of the mercury cathode, but the original cathode of area 10·4 sq. cm. is the most convenient for general use.

Procedure for the electrolysis—Concentrate the neutral or slightly acid solution to 25 to 30 ml. and transfer to a 250-ml., tall-form beaker. Clamp the electrode assembly in place, fill the cathode with mercury to within 1 or 2 mm. of the top and raise the beaker until the electrode almost touches the bottom of the beaker. Add concentrated sulphuric acid until a current of 5 amp. is passing, cover the beaker with a split, notched watch glass, and electrolyse for 15 or 30 min. Add water to maintain constant volume. With the current on, lower the beaker and rinse the electrode with distilled water. Replace the amalgam with clean mercury, electrolyse for 15 or 30 min., and rinse. Repeat the cycle one or more times.

Examples of the use of the technique—A series of samples containing 1 mg. of sodium and from 0·5 to 3 g. of other metal was electrolysed and the resulting solutions were analysed for sodium by the polarographic method (Weaver and Lykken, *Ibid.*, 1947, **19**, 372–376; *Analyst*, 1948, **73**, 169). A blank determination for sodium was performed on the comparable amount of each metal and appropriate corrections were applied. After removal of copper, iron, cadmium, mercury, nickel, and zinc, 1 mg. of sodium was determined with an accuracy of ±0·04 mg. After removal of cobalt, the polarographic method could not be used even though the amount of cobalt remaining in solution was small. The flame-photometer method (Barnes *et al., Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 605–611) was used successfully.

Other separations for which the mercury cathode procedure has been used are, removal of iron before the colorimetric determination of aluminium, removal of iron, nickel, and chromium before the determination of calcium by micro-titration with ammonium hexanitratocerate solution, and removal of interfering metals before the determination of thorium as oxide.

Metals requiring special techniques—Chromium—See (Johnson *et al., Anal. Chem.*, 1947, **19**, 481; *Analyst*, 1948, **73**, 476).

Tin—When a sulphuric acid solution is used insoluble tin salts are precipitated and prevent deposition of tin at the cathode.

Procedure—Dissolve the sample in 20 ml. of concentrated hydrochloric acid, add 2 g. of hydroxylamine hydrochloride, and dilute to 50 ml. Use an electrode assembly fitted at the top of the electrode holder with a fume hood in the form of a wide-angle inverted funnel attached by a side-tube to suction. Electrolyse the solution at 2·5 amp. or less, for 30 min. With the current on, lower the beaker, rinse the electrode, and replace the amalgam with clean mercury. Electrolyse for 30 min., rinse, and repeat the cycle.

The amount of tin left in a solution originally containing 0·25 g. is not less than 3 mg. This amount is not sufficient to interfere in the determination of 1 mg. of sodium.

Lead—The formation of lead peroxide on the anode short-circuits the cell.

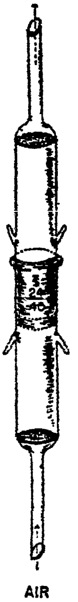
Use a procedure similar to that given for tin. Use 2 g. of hydroxylamine hydrochloride, 0·3 *N* hydrochloric acid, and a current of 5 amp. from the 5-volt supply. The procedure is satisfactory for removing 0·5 g. of lead before determining 1 mg. of sodium.

Molybdenum—With the standard procedure the solution becomes dark and gelatinous. Use a 1·2 *N* sulphuric acid solution and a current of approximately 5 amp. Electrolyse for a total of 90 min., changing the mercury twice. A molybdenum content of 0·1 g. is reduced to less than 1 mg.

Bismuth, antimony, and arsenic—No procedure has been found by which amounts of these metals of the order of 0·1 g. can be quantitatively removed from solution by deposition at the mercury cathode.

B. ATKINSON

Rapid Precipitate Dryer and Solvent Evaporator. I. R. Hunter (*Anal. Chem.*, 1948, **20**, 186)—The dryer is made from two Pyrex funnels of Büchner type with fritted discs. To each of the funnels is sealed a ground-glass joint provided with glass hooks. Funnels with fritted discs 30 to 90 mm. in diameter have been used. Place or filter the sample into the lower funnel, attached to a socket, then attach the upper, inverted funnel, and put rubber bands over the hooks. Pass a slow stream of air upwards through the precipitate for a few minutes. Break up any lumps that may form. Most of the common solvents are completely removed in 5 to 15 min.



To evaporate a solution pour it into the lower funnel while a slow stream of air is passing upwards through it. Attach the upper funnel and increase the air stream.

B. ATKINSON

Review

TECHNICAL DICTIONARY, ENGLISH - ITALIAN, ITALIAN - ENGLISH. By Dr. ING. G. MAROLLI. Second Edition. Pp. 630 + 16 Plates. Florence: Felice de Monnier. 1949. Price L.2700.

It is rather surprising that while there are a number of technical dictionaries in other languages no work of this kind in English and Italian has hitherto been available. The present volume by an engineer of the Fiat company and published in Florence should do much to supply this need.

It comprises terms used in the chemical, engineering, shipbuilding, aeronautical, textile, radio and other industries. The chemical terms, with which the writer is more familiar, seem to be well chosen and correctly translated. That a second edition has been called for within a few months shows that the work has been well received in Italy and the opportunity has been taken to add a considerable number of fresh words in the form of supplements to the two parts of the dictionary.

The printing is clear and free from errors and the volume is of handy size and can be recommended to chemists interested in this subject.

A. H. BENNETT

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.) *To be reprinted.*

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *To be reprinted.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajaput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 1. Determination of Arsenic. *Out of print.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

NEW MEMBERS OF THE SOCIETY

(Elected July 5th, 1949)

George Anderson, A.R.I.C., M.Inst.F.; Alasdair William Armstrong, B.Sc. (Aber.), A.R.I.C.; Laurence Robert Bishop, M.A., Ph.D. (Cantab.), D.Sc. (Birm.), F.R.I.C.; David Geoffrey Elias, B.Sc. (Manc.); Dattatraya Manjunath Gangolli, B.A., M.Sc. (Bombay), F.R.I.C.; Joseph Wylie Gray; Benjamin Crowther James, B.Sc. (Lond.), A.R.C.S., F.R.I.C.; Thomas Moodie, A.H-W.C., A.R.I.C.; Alan Campbell Watson; Ronald Alfred Wells, B.Sc. (Lond.), A.R.I.C.

PHYSICAL METHODS GROUP

The Eighteenth Ordinary Meeting of the Physical Methods Group was held at 8 p.m. on Tuesday, January 25th, 1949, in the Chemical Lecture Theatre of Imperial College, London, S.W.7. Dr. J. G. A. Griffiths, the Chairman of the Group, occupied the chair and there were 70 members and visitors present.

The following papers on "Rheological Methods of Control" were read and discussed (see pp. 387-405): "Industrial Applications of Rheology," by G. W. Scott Blair, D.Sc., Ph.D., F.R.I.C.; "The Application of Rheological Methods in the Milling and Baking Industries," by A. J. Amos, B.Sc., Ph.D., F.R.I.C.; "The Use of Rheological Tests in the Pharmaceutical and Cosmetical Industries," by R. H. Marriott, D.Sc., F.R.I.C.; "Rheological Methods and their uses in the Paint Industry," by P. S. Williams, B.Sc., A.R.C.S.

The Nineteenth Ordinary Meeting of the Group was held at 6 p.m. on Tuesday, February 22nd, 1949, in the Lecture Theatre of the Institute of Physics, London, S.W.1. Mr. B. S. Cooper occupied the Chair and about 110 members and visitors were present.

The following papers on "The Spekker Photo-electric Absorptiometer and Fluorimeter" were read and discussed: "Recent Modifications in the Spekker Photo-electric Absorptiometer," by R. A. C. Isbell, A.Inst.P., who demonstrated the improved form of the instrument; "Some Experiments with the Spekker Photo-electric Absorptiometer and Fluorimeter," by F. Wokes, B.Sc., Ph.D., F.R.I.C., and G. Slaughter.

The Twentieth Ordinary Meeting of the Group was held at 5.30 p.m. on Friday, March 11th, 1949, in the Chemical Lecture Theatre of Imperial College, London, S.W.7. The meeting had been organised by the Polarographic Discussion Panel. Dr. J. G. A. Griffiths, Chairman of the Group, opened the meeting and introduced Dr. W. Cule Davies, Chairman of the Polarographic Discussion Panel, who occupied the chair for the rest of the meeting. About 50 members and visitors were present. The following papers on "Polarographic Analysis were read and discussed: "Buffer Solutions and the Concept of Polarographic Buffer Capacity," by P. Welford, B.A., A.Inst.P.; "Paper Strip Extraction and Polarography," by J. A. Lewis; "The Polarographic Behaviour of Aromatic Nitro Compounds," by J. G. Waller, B.Sc., A.R.I.C.

The Standardisation of Hortvet Thermometers

By R. ASCHAFFENBURG* and J. A. HALL†

(Read at the meeting of the Society on Wednesday, October 6th, 1948)

THE Hortvet technique of determining the freezing-point depression of milk requires the use of special thermometers. Their standardisation with the help of determinations of the freezing-point of 7 and 10 per cent. (w/v) sucrose solutions, a procedure adopted by the Association of Official Agricultural Chemists[1] and endorsed by the Society of Public Analysts and Other Analytical Chemists[2] has rightly been criticised by Stubbs and Elsdon.[3] These authors have recommended the determination of additional reference points with sucrose solutions of intermediate concentrations, so as to narrow the temperature intervals over which correction by linear interpolation has to be applied. Even so, the value of this type of standardisation remains questionable on account of the difficulties encountered when the Hortvet technique is used with sucrose solutions. When water or milk freezes out, the mercury column of the Hortvet thermometer rises to a well defined steady maximum, which is easy to reproduce. With sucrose solutions, on the other hand, the highest point reached by the mercury column tends to vary from determination to determination and is often difficult to observe, as it remains steady for a few seconds only. These difficulties are brought out by the following series of experiments in which the temperature changes in sucrose solutions were recorded at short, regular time intervals.

Determinations were made of the freezing-point of sucrose solutions (Merck, A.R.) ranging from 7 to 10 per cent. (w/v) in concentration, using the standard Hortvet apparatus and technique, except that a platinum resistance thermometer was substituted for the usual mercury thermometer.

The resistance thermometer was comparable in size with the mercury thermometers normally used with the Hortvet apparatus. It had a bulb 6 mm. in diameter and 50 mm. in length, and the combined lag constant of the thermometer and its associated galvanometer was about 5 seconds, which is of the same order as that of a mercury thermometer of similar size.

Readings were made to an accuracy of about ±0·0005° C., and four independent observations on the freezing-point of water, determined in the Hortvet apparatus in the course of the investigation, were concordant to within these limits. Determinations of the ice-point made in the normal manner by immersion of the thermometer in melting ice gave values indistinguishable from those obtained in the Hortvet apparatus.

The temperature observations were made at intervals of a quarter of a minute throughout each determination, except that usually one or two were missed when the temperature was rising rapidly after the super-cooled solution had been seeded. Super-cooling was allowed to reach 1·2° C. before seeding was done and stirring was then stopped. The solution was stirred slowly three times just as the expected freezing-point was approached. The temperature at which this was done is normally indicated by a cross on the graphs of Figs. 1 to 5. In certain instances the temperature was too low to be indicated in this way and then it has been recorded in figures on the graph.

The upper curves of Figs. 1 to 5 show the whole course of each determination and the lower curves record the crucial part of the observation on a much more open temperature scale. The theoretical values given by Stubbs and Elsdon are indicated by broken lines.

The differences between the observed values obtained in the present investigation and the calculated values are plotted against sucrose concentration in Fig. 6. These differences shows a progressively increasing spread of the observations with increasing concentration, and a study of the curves of Figs. 1 to 5 shows that shorter "flats" are obtained on the curves relating to the more concentrated solutions and that, in general, the highest observed values of the freezing-point at any concentration are associated with the longest flats.

Two curves have been drawn to embrace the observations on Fig. 6.‡ The upper curve

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* National Institute for Research in Dairying, University of Reading.

† National Physical Laboratory.

‡One of the observations at 7 per cent. concentration has been excluded as an examination of the form of the curve in Fig. 1 shows that it was abnormal.

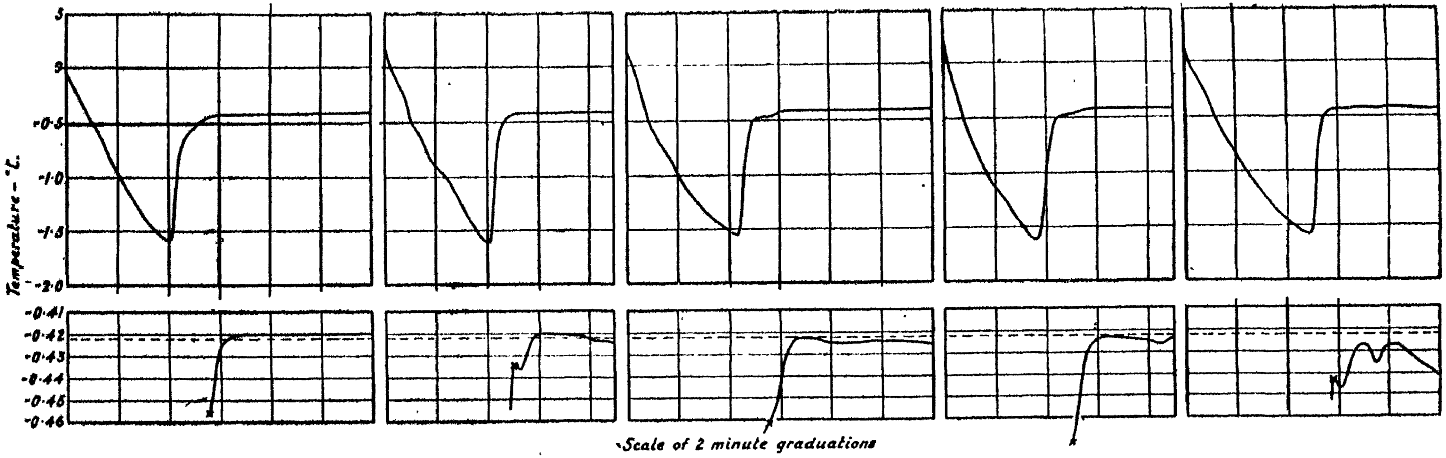

Fig. 1. Freezing points of 7% sucrose solution

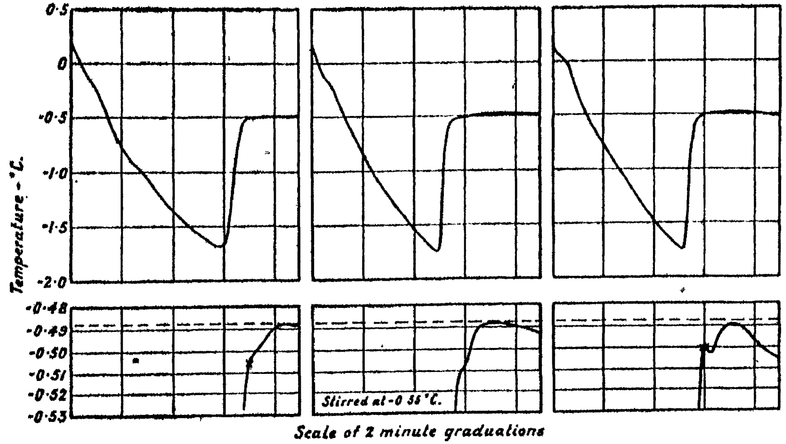

Fig. 2. Freezing points of 8% sucrose solution

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shows that, if the highest observation for each concentration obtained in the present work is taken, it is in agreement with the calculated value to within ±0·003° C. At concentrations up to 8 per cent. inclusive, the result of an individual determination may be considered reliable to ±0·002° C. At higher concentrations, this limit must be widened, as the individual results tend to depart more and more from the calculated values until at 10 per cent. concentration there is a spread of 0·017° C., the observed minus calculated values ranging from −0·002° to −0·019° C. It appears that, beyond 8 per cent. concentration, whilst the highest observed value is not likely to be greatly in error, many observations would have to be made before one could feel reasonably sure that the highest possible value had, in fact, been reached. For example, had one of the observations at 10 per cent. concentration, given in Fig. 5, been omitted, we should have arrived at a value about 0·01° C. too low, and with four observations in quite good agreement at this value.

In view of these uncertainties we cannot endorse the recommended procedure for the standardisation of Hortvet thermometers with sucrose solutions. Instead, we suggest that thermometers should be standardised at a recognised testing institution to an accuracy of at least ±0·002° C. on the International Temperature Scale. In order to cover the essential parts of the thermometer scale adequately, the temperatures at which standardisation is carried out should include values close to +0·050°, 0·000°, −0·400°, −0·500°, −0·520°, −0·540° and −0·560° C. Acceptance of this procedure will facilitate the work of the analyst who will obtain correct results simply by adding to the temperature readings for water and milk the corrections for inequalities in the bore and graduation of the Hortvet thermometer obtained by direct comparison with standard thermometers. This should result in greater reliability of the freezing-point test, and lead to closer agreement between results found in different laboratories.

This paper is published with the approval of the Director of the National Institute for Research in Dairying and the Director of the National Physical Laboratory.

REFERENCES

1. *A.O.A.C. Official and Tentative Methods of Analysis*, 1945, 6th Edition.
2. "The Freezing-Point of Milk," *Analyst*, 1933, **58**, 318.
3. Stubbs, J. R., and Elsdon, G. D., *Ibid.*, 1936, **61**, 455.

THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
UNIVERSITY OF READING
THE NATIONAL PHYSICAL LABORATORY
TEDDINGTON

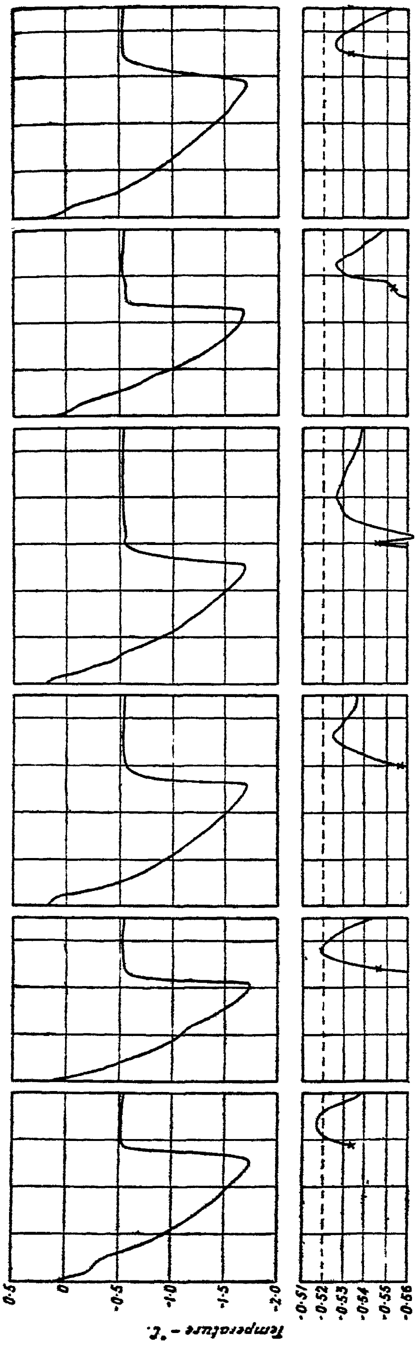
July, 1948

DISCUSSION

MR. R. W. SUTTON, in opening the discussion, said the Society was indebted to the authors for their paper.

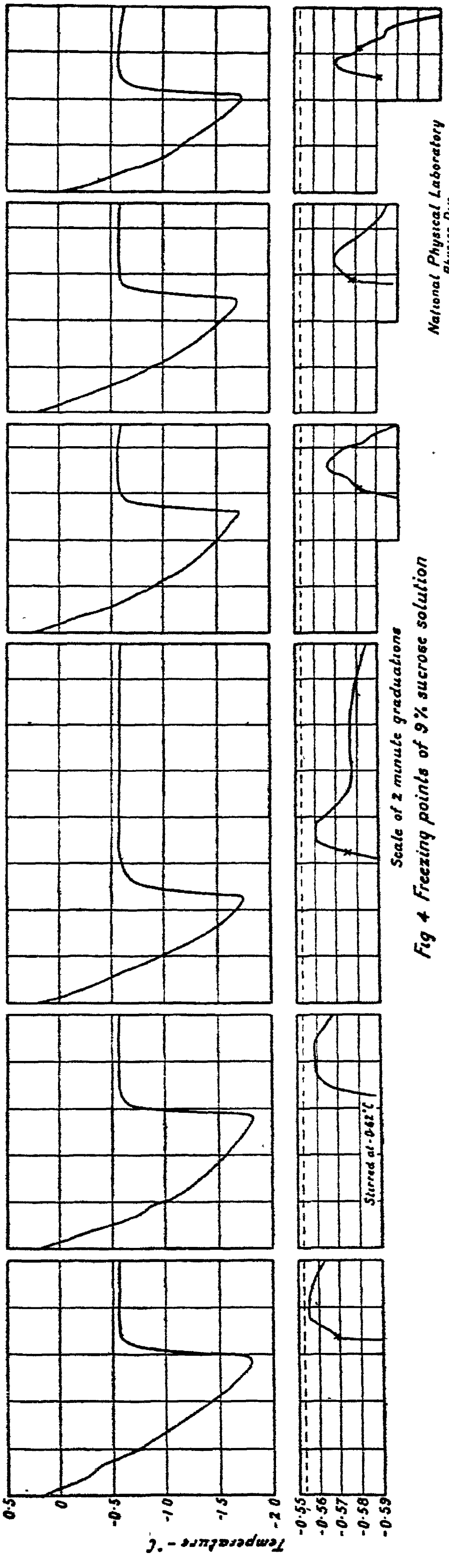
It was important in the first place to make a clear distinction between measurement of the freezing-point of a sample of milk, as accurately as possible, in the Hortvet apparatus and the measurement of a freezing-point by the Hortvet method. He thought that most workers had been concerned with the latter, which involved not only the use of the Hortvet apparatus but also the assumption that a 7 per cent. solution of sucrose in the Hortvet apparatus had a freezing-point of −0·422° C. and a 10 per cent. solution a freezing-point of −0·621° C. These figures might or might not be correct, but they had been accepted as a basis for the standardisation of Hortvet thermometers and any errors in Hortvet's determinations did not fall for consideration.

The difficulty in obtaining reproducible results with sucrose solutions was not new. It had been referred to by Hortvet himself, and later by Elsdon. Other workers were aware of the difficulty, and in his laboratory it had been the practice for many years to make a considerable number of readings with 7 per cent., 10 per cent. and intermediate concentrations of sugar in a full standardisation every two or three years and to check more frequently with 8·5 or 8·75 per cent. sugar solution. After a comprehensive standardisation it was possible to use the A.O.A.C. method of calculation for a smaller interval or to construct a table giving the appropriate "stem" correction to be applied to any reading. He felt that the authors had to some extent exaggerated the difficulty with sugar solutions, and he could not agree that the difficulty was encountered only with the stronger solutions. In his experience there seemed to be a direct correlation between the variability in the results and the concentration of the sugar solution. It must also be remembered that in the A.O.A.C. method any errors in the work with sugar solutions are not included in full and with cumulative effect in the final result. In fact, if the error with the 7 per cent. sugar solution is negligible, only about one-half of any error in the figure for the 10 per cent. solution would be included. Further, by using intermediate strength sugar solutions, the freezing-points for which had been calculated



Scale of 2 minute graduations

Fig. 3. Freezing points of 8 5% sucrose solution



Scale of 2 minute graduations

Fig 4 Freezing points of 9% sucrose solution

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by interpolation from Hortvet's figures for the 7 and 10 per cent. sugar solutions, it was possible to reduce the error to quite low proportions at a point where the greatest accuracy was required, *i.e.*, −0·530° C.

In his opinion these proposals for a different procedure in the Hortvet test ought to be adopted only if they led to better agreement in results obtained on the same sample by different workers, and only if they did not involve any change in our standards. He was at present doubtful if either of these conditions would be satisfied, but this could only be determined by further experimental work.

On the first point it appeared that the N.P.L. would guarantee their corrections to ±0·002° C. On this basis a freezing-point depression (zero minus the milk reading) might be incorrect by ±0·004° C. To this possible error (entirely a feature of the thermometer and its certificate) must be added the normal experimental error, which he thought might reasonably be put at ±0·001° C. or possibly ±0·002° C. We then arrive at the fact that the results for a sample of milk examined by different workers might conceivably range over 0·010° to 0·012° C. This range would apply at any level and would be just as likely in measuring a depression of 0·530° C., where the greatest accuracy was required, as in measuring one of 0·470° C., where great accuracy was unimportant. It was his opinion that better results than this could be achieved by careful work with sugar solutions, and that by using the intermediate strength sugar solutions the error could be kept low at the point where the greatest accuracy was required.

On the second point he emphasised that many valuable records had been compiled over a period of years, and it was a fair statement that 0·530° C. could be accepted as the minimum depression obtained in the Hortvet method for genuine milk. Evidence to this effect had been given in the Courts on many occasions and it would be an unfortunate complication to have to alter this figure because of a new procedure for the standardisation of the thermometer. Hortvet's figures for 7 and 10 per cent. sucrose solution may not have been correct, and indeed it seemed to him quite likely that using the most accurate thermometers different results might be obtained in different Hortvet cryoscopes, owing to certain features in the apparatus itself, which in his opinion were defects when close measurements of temperature with a mercury-in-glass thermometer were to be made. The stirring was inefficient and variable according to the manipulation of the stirrer; the heat that was liberated was therefore expended in rather a variable way in raising the temperature of some parts of the apparatus and the solution under test, and all the time the outer cooling-bath, maintained at a lower temperature, was exerting a cooling effect. He had always thought that the move to standardise on sugar solutions represented an advance in that it tended to eliminate some of the variables that were peculiar to the particular apparatus being used.

There was no inherent defect in the use of sugar solutions. In his opinion the defect was in the Hortvet apparatus. He had recently made further experiments with Monier-Williams's apparatus and the contrast was marked. In using this apparatus, when the appropriate degree of super-cooling had been reached, the freezing-point tube was isolated from the cooling-bath (by the removal of the dilute alcohol between the two tubes) and the results with the 10 per cent. sugar solution were quite steady and quite consistent.

Lastly, the manner in which the results were expressed was for practical purposes quite unimportant There was no disadvantage in using the arbitrary scale of the Hortvet method and there was no particular merit in using the International Temperature Scale. The temperatures recorded were not true freezing-points, as there was no correction for heat exchange or the amount of super-cooling. The figures, however they were recorded, were quite arbitrary ones obtained in a particular apparatus and following a particular procedure.

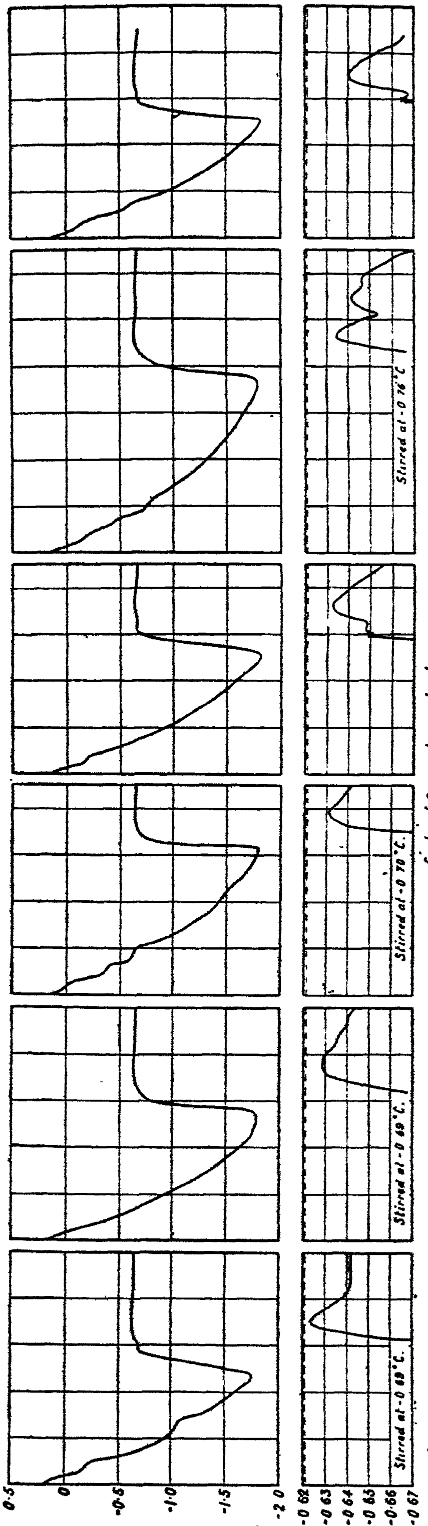
He thought these proposals deserved very careful attention, but that before they were adopted there ought to be ample experimental evidence that they would enable us to obtain more consistent results and above all that there would not need to be any alteration in the figures that had been accepted for so long as typical of genuine milk.

Mr. J. **Markland** said that Dr. Aschaffenburg had mentioned that there was no means of knowing when the freezing-point determination of a sugar solution should be accepted as correct, but the A.O.A.C. specified that a determination should be rejected unless the top of the mercury column remained stationary for at least 1 minute.

He enquired if Mr. Hall could say how the heat capacity of the platinum resistance thermometer compared with that of a mercury-in-glass Hortvet thermometer. If the heat capacities were different, the amount of ice formed on freezing might also be different. The consequent effect on the freezing-point might, or might not, be negligible, but a different heat capacity was a theoretical defect in the method of checking Hortvet's figures for the 7 and 10 per cent. sugar solutions by use of a platinum resistance thermometer. This point could possibly be checked by determinations of the freezing-point of the same sample of milk, using on the one hand a platinum resistance thermometer and on the other hand a Hortvet thermometer standardised by the N.P.L. method.

Mr. H. F. **Bamford** pointed out that milk was a colloid and one might, therefore, expect that the molecular concentration and the freezing-point would be less altered by the separation of a small amount of ice than would that of a sucrose solution of the same freezing-point. The choice lay between two sets of errors, one occurring when each operator determines his own standard and the other when an accurately calibrated thermometer is used. Which of these procedures would be likely to lead to better agreement between different laboratories could only be decided by a statistical examination of the two sets of errors.

Mr. N. **Heron** thought that undue emphasis was being given to 10 per cent. sucrose solution, the freezing-point of which, −0·621° C., was well below the range for normal milks. The lowest point reached



Scale of 2 minute graduations

Fig. 5. Freezing points of 10% sucrose solution

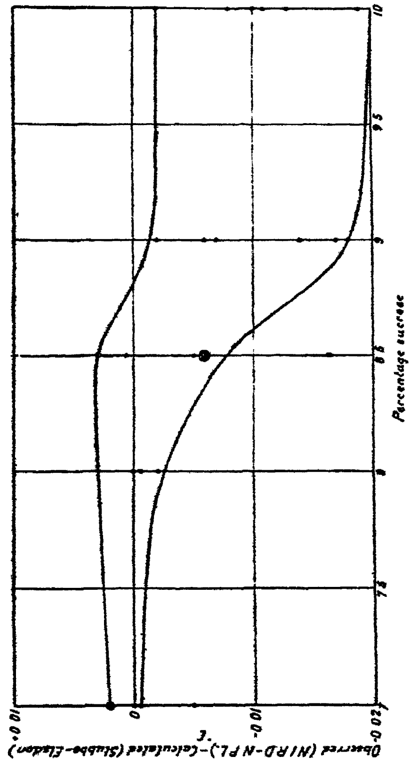


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Fig. 6. Freezing-points of sucrose: departure of observed from calculated values

by these was of the order of 0·550° and this was covered by 9 per cent. sucrose solution. The suggestion that satisfactory freezing-point determinations could not be obtained with sucrose solutions up to 10 per cent., however, was not borne out by the work of Elsdon and Stubbs nor by later work of many other analysts. In his opinion it was more satisfactory to standardise the thermometers in the laboratory in which they were to be used than to rely on external references, however reliable.

Dr. J. H. Hamence wished to thank the authors for bringing this matter before the Society and also for the work that they had done on what was undoubtedly a difficult matter.

He regretted very much the suggestion made by the authors that Hortvet thermometers should in future be standardised by the N.P.L. and that all the analyst should be required to do would be to confirm the zero point of the instrument with water. This procedure was a complete breakaway from the original Hortvet technique; he agreed that the N.P.L. would be doing a great service to analysts by checking the bore of the thermometer, but he was firmly of the opinion that the standardisation of the thermometer should still be done against a sugar solution. The authors had shown that a satisfactory standardisation could be made with a 7 per cent. sugar solution. Why, therefore, could not this be used to check the thermometer in the Hortvet apparatus?

The result of a freezing-point determination frequently led to legal proceedings and he felt that it was wrong to give evidence in court on an instrument that he himself had not standardised. This difficulty would be obviated if the thermometer were checked against a sugar solution.

It must be borne in mind that the Hortvet method was a purely empirical one and, therefore, in his view, it was important, particularly having regard to the wide variation that exists between different pieces of apparatus, that a sugar solution should be used for standardisation.

Mr. Hall, in reply to Mr. Sutton, said that it was of course possible, when either end of a range of temperature was specified to an accuracy of ±0·002°, that there might occur an error of ±0·004° in the interval. On an N.P.L. certificate, however, these limits were intended to include any accidental errors in the reproduction of the International Temperature Scale by the N.P.L. standards, and such errors were very unlikely to change from positive to negative over a short temperature range. Hence, in practice, it was extremely improbable that the intervals obtained from a certificate would be anything like so much in error. In fact, it was to enable differences to be measured to a higher degree of accuracy than absolute temperatures that the corrections on the certificate were always recorded to a higher degree of accuracy than the plus or minus limits. For example, when the limit of accuracy of test was stated to be ±0·002°, the corrections were recorded to the nearest 0·001°.

He would like to emphasize that, if the best observations only were taken, there was no difference between the scale defined by sucrose solutions and the International Temperature Scale. The difficulty was to know when one had got a really reliable observation with the more concentrated solutions. In fact, the use of sucrose solutions was simply a more roundabout way of attempting to reproduce the International Scale. Hortvet determined the freezing-points of the sucrose solutions on the International Scale by using thermometers standardised on that scale by the Bureau of Standards. Hence, if other thermometers were standardised in sucrose solutions, accepting Hortvet's values and assuming that the experimental work was ideally accurate, they should indicate temperatures on the International Scale. It was obvious, however, that there was less chance of experimental error if the thermometers were compared directly with the standards of a laboratory such as the Bureau of Standards or the N.P.L., than if the intermediary of sucrose solutions was employed. But, if the standardisation work by the two methods was satisfactory, the change from one to the other would not lead to a change in the numerical value of 0·530° for the minimum depression of genuine milk, so that the old values for milk freezing-point depressions would still hold good.

In reply to Mr. Markland he would say that the heat capacity of the bulb of their resistance thermometer would certainly be less than that of the normal Hortvet mercury thermometer, but he would not like to guess what effect any difference would have on the results. Did any marked difference in the weight of the freezing-point tube or of the stirrer have any effect? If the result was reasonably independent of variations in these other neutral masses, he would not expect it to vary with the heat capacity of the thermometer.

Dr. Aschaffenburg, in reply to Mr. Sutton, said their observations had shown that, with the more concentrated sucrose solutions, one might obtain a series of temperature readings in close agreement, but definitely on the low side. Unless an analyst had a certificate of standardisation, he had no criterion that would enable him to conclude that the readings, though in good agreement, were in fact erroneous.

In reply to Mr. Markland, he said it was true that the A.O.A.C. defined a satisfactory determination as one in which the top of the mercury column remained stationary for at least 1 minute. The trouble was that, with 10 per cent. sucrose solutions, for instance, one could hardly ever get a result at all on this basis. As the data now presented showed, only a very short stationary temperature maximum was obtained with the more concentrated sucrose solutions.

In reply to Dr. Hamence, he said that the point obtained by checking with 7 per cent. sucrose solution was too far removed from the essential milk range to allow of extrapolation to that range. It was necessary to have at least one other point, obtained with a more concentrated sucrose solution, to find out what the error was in the region of 0·530°. He would like to emphasize again that their best observations were in agreement with the values given by Hortvet and by Stubbs and Elsdon. He did not think they were departing from what had been done in the past, except that they suggested a transfer of the onus of the basic standardisation from the analyst to the experienced thermometry experts at the N.P.L.

Rheological Methods of Control

The following four papers were read at a meeting of the Physical Methods Group on Tuesday, January 25th, 1949

Industrial Applications of Rheology

By G. W. SCOTT BLAIR

The accepted definition of the term "rheology" is "the science of the deformation and flow of matter." This includes a very large part of physics but, by convention, certain sections, such as hydro- and aero-dynamics and parts of the theory of elasticity, when not concerned with the flow *properties* of materials as such, are excluded.

There are many reasons why it may be of practical importance to measure the flow and deformation of raw materials, intermediates or finished products in industry: some of these reasons are well illustrated by the authors of the other papers in this symposium.

As an illustration from a raw material, it is not possible to mill wheat satisfactorily if the berries are too hard or too soft for the "break," and one purpose of wheat conditioning is to attain the correct moisture content and distribution so as to ensure the proper balance of brittleness and plasticity. For some materials, such as this, a rapid moisture determination* may be more practicable than a mechanical or physical test; but in others, as in assessing when the proper amount of whey has drained out syneretically from cheese curd in the scalding process in making hard-pressed cheese, a superficial density is more readily measured than a moisture content.[1] Occasionally, an indirect rheological test may be convenient when purely chemical information is required, as when the viscosities of certain fats and fatty acids are found to be highly correlated with their iodine numbers.[2]

There is, then, no particular necessity to use chemical analysis when chemical constitution is of direct importance and physical methods when mechanical behaviour is in question so long as the level of correlation between chemical constitution and mechanical behaviour is known and is sufficiently high. In general, it may be said that rheological analysis is seldom as accurate as chemical analysis, but is often considerably quicker and easier to carry out in the factory.

With finished products, rheological properties may be important for a variety of reasons. These may be classified as follows—(1) *Direct mechanical requirements* of the product, *e.g.*, adequate strength of a plastic, correct consistency and thixotropy of a drilling mud, brushing qualities of a paint; (2) *Indirect mechanical requirements, e.g.*, the "body" of a cheese determining the preponderance of different types of micro-organism and so, indirectly, of flavour, taste and keeping quality; (3) *Requirements of public taste, e.g.*, in cosmetics (see Marriott's paper in this symposium); (4) *Uniformity requirements*, the consumer often preferring a uniform consistency to a variable consistency even if the latter includes occasional improvements; (5) *Mixed requirements, e.g.*, spreading capacity of butter, which is, within limits, a matter of public taste but in extreme cases a "direct mechanical requirement" in relation to the crumbliness of the bread.

Although there are a number of rather ill-defined rheological properties that are not directly concerned with stress - strain - time (S : σ : t) relations, most rheological requirements may be said to depend on the functional relationships between these three variables.

The very definition of rheology suggests two principal types of behaviour: flow, in which the changes of form of bodies are functions of both stresses and time; and deformations which, while dependent on the system of stresses, are often immediately produced and can be recovered when the stresses are removed. The system of stresses can be defined in terms of a symmetrical second rank tensor with six independent components and the deformations, or rates of deformation, also by other tensors with six independent components. In the classical case of an elastic solid, the coefficients relating the components of stress and strain themselves form a fourth rank tensor which, being symmetrical, cannot have more than 21 independent components. For materials like wood, which are said to be "orthotropic," this number reduces to nine and, for perfectly isotropic materials, to two.

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* Though even this is often done indirectly by measuring some physical property such as dielectric constant.

For a simple viscous fluid only one independent coefficient is normally required to specify the relationships between stress and rate of flow, though it must not be forgotten that the complete story is not nearly as simple as this statement might seem to suggest.[3]

Happy indeed is the industrial rheologist who can work with materials for which such comparatively small numbers of $S : \sigma : t$ properties are invariant. Frequently, a choice must be made between measuring simple physical properties, such as viscosity or elastic moduli, under conditions quite unlike those obtaining in practice, and designing empirical tests which imitate the processes that the material undergoes in manufacture or use or the way in which it is handled when judged subjectively by the expert.

Indirect tests, though occasionally very valuable (*e.g.*, the good correlation between the viscosity of certain solutions of cotton[4] and silk[5] and the resistance of the materials themselves to chemical attack), are more often dangerous and misleading. Early attempts to correlate "strength" of flour doughs with the viscosities of dilute aqueous suspensions of flour, and later with the size and strength of bubbles blown in dough,[6] illustrate the dangers of such indirect methods unless used under strict statistical supervision.

In studying the behaviour of complex materials like doughs, pastes and magmoids, it is usual to keep either S, $\dot{S}$, σ or $\dot{\sigma}$ constant,* measuring the changes of whatever is free to change, with the course of time. Ingenious devices for working at constant S[7,8,9,10], $\dot{S}$,[11] σ[12] and $\dot{\sigma}$[13] have been described, though too often there have been minor but important errors in design or calculation.[14]

The interpretation of data from such tests is always difficult. Some years ago it was usual to suppose that complex behaviour must be due to the interaction of a number of essentially simple factors, *i.e.*, that rheologically complex systems are actually built up of groups of molecules, some of which behave as simple elastic units ("springs"), some as truly viscous units ("dash-pots"), some as sliding frictional units, and perhaps some as ratchet mechanisms which inhibit the recovery of potentially stored energy. These ideas led to the construction of many ingenious and complex models which, in a few instances no doubt, helped to give an understanding of the molecular structures involved, the units being associated with different components of the system (*e.g.*, Schofield and Scott Blair's work on the protein and starch components of flour doughs.[15])

But in most instances it came to be realised that the use of such models meant no more than the empirical fitting of exponential equations to the data: the models could equally well be replaced by combinations of electrical resistances, capacities, etc. The use of such exponential treatments is often fully justified but the models become unnecessary in any case, and often better and simpler agreement with the experimental data is got by using other types of equation.[16]

It is, of course, almost always *possible* to describe the experimental data in exponential terms, provided enough terms are used, but there has been a tendency of late among those who still use rheological models to introduce imperfect or at least non-linear elastic springs, and "dash-pots" containing non-Newtonian liquids as an alternative to very large or even infinite numbers of units.[17] It is generally claimed for models that they enable complex behaviour to be described in simple terms. The best that can be said for such models is that they describe complex behaviour in perhaps rather less complex terms.†

In experiments in which stress relaxation is measured at constant strain, not only is it often necessary to introduce a number of truly Maxwellian units (*i.e.*, exponentially relaxing units) but very frequently a distribution spectrum of an infinite number of such units is postulated. (This type of treatment goes back to Wiechert's work in 1893. For a good modern account, see Simha.[18])

Maxwell[19] himself, however, when he first proposed the concept of "relaxation time," warned his readers that these relaxation times might well prove to be a complex function of stress, and it is indeed true that in the majority of materials, at least at room temperature, the relaxing stress is much more simply related to time by equations of quite a different type.[20]

It must be realised that, more often than not, rheological tests on complex materials are made under conditions which are not those of equilibrium nor do the "properties" of the material remain unchanged as a result of the process used to measure them. There is a type of "uncertainty" here as significant, in its way, as the famous "uncertainty" involved in measuring the position and momentum of an electron. To measure a flow or a deformation, one must first produce a flow or deformation; and these, or the stresses associated with them, may well change the material even as it is being tested; so that we are in fact measuring a process rather than a property.

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* A dot above a symbol indicates differentiation with respect to time.

† This type of treatment is closely parallel to the use of non-orthogonal factors in Factor Analysis by Thurstone and others—see below.

The advantage of the classical "property" lies in the fact that it is invariant to the process used for its measurement, for example, the viscosity of simple Newtonian liquids is the same whether determined in a rotation, a capillary or a falling ball viscometer. Unfortunately, many materials do not behave in this simple manner. Some show regular Newtonian behaviour in a falling ball viscometer but are non-Newtonian in a capillary.[21] Others show complex behaviour in all types of viscometer, and it is often best to describe their behaviour in terms of "quasi-properties." Such quasi-properties form a hierarchy: in some cases the processes which they measure can be produced by a variety of experimental methods; in others, though replicable* with fair accuracy provided that the same testing method is used, they are not invariant to the method of test. It is the writer's personal opinion that, where no invariant "properties" exist and especially under non-equilibrium conditions, quasi-properties, at least those of a high order, form the best concepts with which to describe the rheological behaviour of many complex systems. This opinion is based on the results of about a thousand experiments on a very wide variety of materials.

Ordinary properties such as viscosity are described in terms of a single quantity. Quasi-properties require also one or more (usually two) quality factors which indicate the degree to which the material approximates towards the simple prototypes. These groups of magnitudes, comprising normally a quantity and two quality factors, must be treated as wholes in the description of materials. The "quantity" alone, for example, gives no means of comparing materials. Failure to realise this has given rise to unjustifiable criticisms of the whole treatment on dimensional grounds.† These criticisms would have been partly justified had the quasi-properties been split up,[23] but this obvious mistake does not seem to have been made.

The theory of quasi-properties has also the advantage that it links the physical rheological tests with the judgments of experts who assess quality by handling materials.

So long as physical theory remains as incomplete as it still is, it is certain that many industries will continue to rely for their rheological testing mainly on the subjective judgments of skilled craftsmen.

It is useless merely to deplore this—just as it is useless to deplore the lack of simple physical properties. Both facts must be faced and detailed studies have been and are being made of the psycho-physical processes involved in assessing the rheological behaviour of materials by handling them and of the nature of the craftsmanship involved.[14]

In classical rheology we have seen that we are concerned with stresses and rates of change of stress, and with strains and rates of change of strain, *i.e.*, with whole-number differentiations. Psycho-physical experiments strongly suggest that the entities by which "body," "spring" and so forth are judged when materials are handled, are fractional differentials of strain with respect to time and perhaps stress, and the theory of quasi-properties is based mathematically on similar fractional differential equations.

It is certain that the judgment of the rheological behaviour of materials in handling them depends not on isolated "physical properties" but on "*Gestalten*" which are closely related to the quasi-properties just described.

It has been objected that such concepts are unlikely to further our knowledge of the underlying molecular mechanisms of rheological processes. This is freely admitted, but it is equally strongly maintained that the elucidation of molecular mechanisms is far from being the sole purpose of rheology.

There is another type of entity by which the complex rheological behaviour of materials may be described when simple rheological properties are not measurable, nor is it yet known whether this new treatment links up effectively with the theory of quasi-properties.

In many industries a comparatively large number of empirical tests are performed as a matter of routine on products or intermediates. Some of these tests are rheological, some chemical, some electrical: some measure simple "properties," others do not. Very often it is not known precisely what the test measures nor how far the results of one test overlap with those of another. The latter point is, of course, determined by calculating the correlation coefficients between the tests and, if all these are calculated, a square matrix can be written comprising all the tests. But each sample is still virtually described by as many magnitudes as there are tests (say n), even though it is known that all or most of the relationships between the measured behaviour could be accounted for by considerably less than n magnitudes.

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* By "replicable," as distinct from "reproducible," is meant that the measurements can be repeated satisfactorily on replicate samples, but not reproduced on samples which have already been tested.

† For a defence of the treatment, see Dingle.[22]

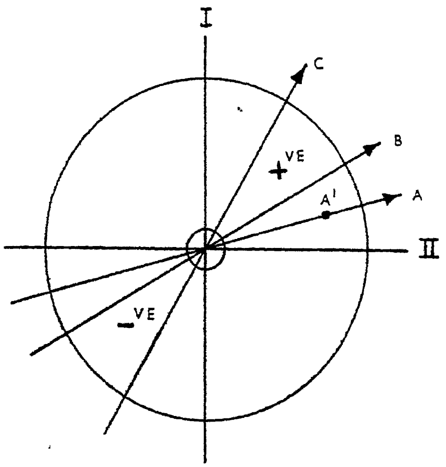
In simple cases the behaviour is, of course, described by $m(< n)$ "physical properties," but in most of the materials of interest to rheologists this cannot be done, since no invariant "physical properties" can be found.

The problem is, then,.to reduce a space of n dimensions to one of $m(< n)$ and preferably to choose m in such a way that its co-ordinates are independent of one another, *i.e.*, "orthogonal."

A very similar problem has arisen in psychology in an even more acute form, since large batches of psychological tests are often used on a big population, and there is no independent way of expressing in objective terms what it is that each test measures.

The treatment of this problem is revolutionising psychometry and has probably done more towards its establishment on a scientific basis than any other single development. It appears likely that factorial analysis or some similar statistical method may simplify very greatly the handling of complex industrial rheological data. Preliminary experiments on cheese[24] and plastics[25] give much cause for encouragement.

There are a number of ways in which factorial analyses can be carried out.[26] Only a brief account of the method can be given here. Suppose, for the sake of simplicity, that we wish to reduce the data from a number of tests in terms of two independent factors. The tests may be represented as straight lines passing through a common origin (see Figure).



Three such tests, A, B and C, are shown in the figure, the correlation coefficients between them (r_{AB}, r_{BC}, r_{AC}) being given by the cosines of the angles between the lines. It is clear that each test requires two magnitudes for its complete description on this diagram* and, since we have chosen to use two-dimensional space, the tests may be represented as vectors and are therefore marked with arrow-heads.

The use of only two dimensions, that is, a reduction to only two factors, is, of course, an over-simplification. No doubt other factors will be required for the complete description of the data, nor are the tests without experimental error. The circle is really part of a sphere, if not of a hypersphere, and the fact that the tests are displaced into at least a third dimension means that the projections of the displacements will bring the test-points into positions intermediate between the origin and the periphery of the circle in our figure. Such a point is shown at A′. Also, the angles between the vector lines will no longer have for their cosines the simple correlation coefficients.

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* A vector requires for its specification as many magnitudes as there are dimensions. If we reduce our tests in terms of m factors, we shall require m magnitudes for their specification.

If we scale each test so that the radius of the circle is equal to the standard deviation, then the area $[O\,A']^2$ is a measure of the extent to which our two-factor solution accounts for the variance and is called the "communality." The value of $1-[OA']^2$ is called the "uniqueness."

In the correlation matrix, instead of the more obvious procedure of filling the diagonal cells with 1·0's (since the correlation of any test with itself is presumably unity) we insert here the communalities, thus making allowance for errors and for any factor unique to the test itself. It is this procedure, which has to be done by trial and error, that makes the analysis rather lengthy, but there is, as yet, no way of avoiding it. If "ones" are used in the diagonal cells we shall not reduce our number of dimensions.

Any pair of axes at right angles, such as those marked I and II in the figure, can serve as independent factors (since $\cos 90^\circ \doteq 0$) and the choice of the position of the principal axis is in our power. By the use of matrix algebra we can, of course, handle any number of factors which would require hyperspheres for their geometrical representation. But for those who are helped by models, three-dimensional "cross-sectional" models are easily made.

It will be appreciated that we may take wide liberties not only with the sign but even with the scaling of our tests. An instrument measuring some rheological property, say hardness, on a "linear" scale might equally well and more meaningfully have been designed with a reciprocal ("softness"), a square or a logarithmic scale. But if we change the scaling of a test, care must be taken to ensure that correlations are improved throughout the whole matrix.

The factor matrix itself (F) is derived from the equation $R = F.F'$. The correlation matrix, R, is of course square: F and its transpose F' are rectangular, the divergence from squareness representing the amount of saving which the factor analysis has been able to effect.

Where very large correlation matrices are available, or if there are artificial limits to the parts of the distribution curve in which samples may be found, it is sometimes advantageous to make at least a preliminary analysis in terms of non-orthogonal axes, a procedure which, as stated above, is similar to the use of non-Newtonian dash-pots and non-Hookean springs. In the writer's opinion, such methods should serve only as a first stage and, if models are to be used, the treatment should be followed up by making simple models of the units of the complex models.

So far as the writer is aware, he and his colleagues are the first to take up Thurstone's suggestion[26] that factorial analysis should be applied to purely physical processes. A comparatively large proportion of the present paper has been devoted to this method because it is our belief that its use will prove of great practical importance in the interpretation of empirical rheological tests and perhaps in developing theories of the behaviour of complex rheological systems.

I am indebted to my colleague, Mr. R. Harper, for reading through my short account of factorial analysis—a technique with which he is much more familiar than I am.

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Rheological Methods in the Milling and Baking Industries

BY A. J. AMOS

WHEATEN flours contain two main proteins, gliadin and glutenin, which in the presence of water form a complex, known as gluten, possessing both elastic and plastic properties. In a properly made dough the gluten takes the form of an interwoven network of fibrils which constitutes the skeletal structure of the dough mass. The nature of this network, and hence the number and nature of the individual gluten fibrils, must be such that the dough can be inflated with gas to a suitable degree without serious distortion of its moulded shape and can continue to exhibit a fine and even vesiculation internally. It is no wonder, therefore, that from the very early days of cereal science attempts have been made to evaluate the physical properties of gluten. Since 1886, when the first attempt was recorded, well over thirty different rheological methods have been devised for assessing the breadmaking quality of flour. Many of these are of only historical interest, and of the others only about half a dozen have gained popularity.

None of the few well known dough testing instruments in use in cereal laboratories to-day is the outcome of a fundamental approach to the problem. By that I mean that the designers of these instruments did not first ascertain the significance of certain strictly definable physical attributes of dough behaviour and then devise suitable methods of measuring these attributes. Rather did they evolve a technique for obtaining mechanically in graphical form reproducible representations of stress/strain relationships imposed upon a dough and then interpret the curves thus obtained in the light of the observed breadmaking quality of a replicate dough. If often happens, therefore, that a particular measurement derived from the curve furnished by an accepted dough testing instrument is not an index of a strictly definable and individual physical property, and indeed it may even not be possible to express in absolute terms exactly to what the measurement does refer. On the other hand, the measurement will have a very real meaning in terms of dough behaviour, and this is why these instruments have proved such valuable tools to the cereal chemist. Within the last decade, however, excellent work by investigators in this country has established the correlation between certain aspects of baking quality and definable physical properties of doughs. These workers have devised methods of measuring these physical properties, but commercial models of their instruments are not yet available.

The dough testing instruments which are empirical in principle but are widely used are of two main types. There are those in which the resistance of the dough to a given mechanical mixing operation is recorded graphically throughout a continuous mixing of 5 to 20 minutes, and the other type in which the dough is stretched until it breaks and throughout this operation, which takes only a matter of seconds, the stretching force and the corresponding amount of stretch are recorded in graphical form. In this country the best known instrument of the former type is the Brabender Farinograph (Fig. 1). The essential features of this machine are a twin-bladed, water-jacketted mixer of special design and a directly coupled electric motor, which is supported so that it is free to rotate in a vertical plane and to the housing of which is connected a pen that can write upon a clock-work driven band of ruled paper. When a dough is being mixed in the mixer of this instrument, the resistance that it offers to the movement of the mixer blades causes a rotational movement of the suspended motor and a corresponding movement of the pen. The type of curve furnished by this instrument is shown in Fig. 2. The mean height of the band that the pen produces is directly related in the early stages of the mixing operation to the proportion of water that has been added to the flour. Hence, if the height of the band which corresponds to the dough consistency desired by the baker is known, this instrument can be used to determine the water absorption of a flour. As the mixing operation continues the height of the band produced by the pen decreases owing to the effect of the continued mechanical abuse of the dough.

The curves produced by this instrument do not provide numerical assessments of individual physical properties of the dough, but they are capable of furnishing data that are correlated with certain aspects of dough behaviour of importance in the breadmaking process. Thus, the distance AB is a measure of the time needed under the conditions of the test to attain proper dough development. Flours that exhibit a prolonged dough development time are generally what the baker calls "strong" flours and will usually give good results in high-speed mixers. Sometimes, however, a prolonged dough development time may arise from an unusually slow rate of imbibition of water by a protein of only fair quality. The distance BC, which represents the time during which the curve continues at the initial level, is regarded as a measure of dough stability. The distance DE, which is the units of consistency lost during a given period of mixing, is termed the "dough weakening time" and is an index of the ability of the flour to withstand fermentation and mechanical abuse.

In Fig. 3 appear the Farinograph curves of flours of very different breadmaking qualities. It will be seen that the flours from the Manitoba wheats, which contain a high quantity of good quality protein, give curves indicative of very great stability. The curves of the flours from the Plate wheats show much less stability and the smaller widths of these bands agree with the less springy or elastic natures of their doughs. The short development time and poor stability exhibited by the bottom right-hand curve are characteristic of the curves of weak flours more suitable for confectionery work than for breadmaking.

The principle upon which the Farinograph operates permits tests to be made with fermenting doughs. In such tests it is customary to run the mixer until the dough is properly developed (normally 3 to 5 minutes), and then to give the dough a further mixing for 2 minutes at hourly intervals during a fermentation period of, say, 5 hours. This procedure furnishes curves such as those depicted in Figs. 4 and 4*a*. It will be seen from these figures that such curves do provide information upon the ability of a flour to withstand fermentation and can give some indication of the period at which the dough should attain correct ripeness.

Other forms of recording mixers which furnish curves similar to those produced by the Farinograph are in use in America, although they have not been adopted in this country. A good example of these is the Mixograph, which is shown in Fig. 5. The mixing head carries four vertical pins which move among four fixed vertical pins in the mixing bowl with a planetary motion when the head is lowered into position. The tendency for the bowl to rotate as a consequence of the pressure exerted on its fixed pins is impeded by a standardised spring in a manner that permits the torque to be recorded. Curves produced by the Mixograph are shown in Fig. 6.

In this country and on the Continent, wide use is made of two instruments of the other type, in which the dough is stretched until it breaks and both the stretching force and the degree of extension are mechanically recorded during the stretching operation. The older of these two is the Chopin instrument; initially it was known as the Extensimeter, but the improved model, which has been developed during the last ten years, is known as the Alveographe. In this instrument, shown in Fig. 7, a disc of non-yeasted dough of fixed dimensions is clamped across an air inlet and then blown into a bubble until it bursts; the varying pressure within the bubble is depicted throughout the operation by a recording manometer. The dough is mixed, moulded and cut into discs under rigorously standardised and controlled conditions and throughout the inflation procedure its temperature is kept constant.

There is a radical difference between the technique of evaluating dough properties by means of the Chopin Alveographe and by the Farinograph. In the Farinograph method all doughs are made to the same consistency and a preliminary test is always performed in order to determine the proportion of water that has to be added to the flour to arrive at the standard of consistency that has been adopted. The doughs used in the Alveographe, on the other hand, vary greatly in consistency, but they are constituted so that in each of them the ratio of total water, *i.e.*, added water plus water natural to the flour, to dry solids is a constant.

Three curves furnished by the Alveographe are shown in Fig. 8. The measurements usually made from these curves are three. The height of the peak is measured as an index of the stability of the dough, the length of the base of the curve is, of course, a measure of its extensibility, and the area enclosed by the curve is a measure of its "strength." Although the mathematics of this technique and the curves it furnishes are very complicated and it is doubtful whether the curves provide a numerical assessment of any single property, this instrument does provide extremely useful information upon the nature of the protein of a flour. It is particularly valuable as a means of evaluating the blending potentialities of wheats, where what is required is a knowledge of the inherent protein characteristics of the wheats rather than information upon how they will behave in a commercial flour when subjected to the influence of enzymic action and of chemical improvers. The use of the Alveographe for evaluating wheats is well illustrated by the curves in Fig. 8. The top left-hand curve is that of a flour with good breadmaking qualities. It has a high strength and is well balanced; it would give a dough with properties pleasing to the baker which would stand up well to mechanical treatment in the bakery. The flours represented by the other two curves are identical in strength with the first flour, since the areas of the three curves are identical, but they are quite unsuitable for breadmaking. The top right-hand curve reveals excessive stability and very poor distensibility, corresponding to a clay-like dough. The bottom curve indicates poor stability but excessive distensibility, in other words, a soft "flowy" dough. The importance of the balance between the individual physical attributes, and hence the value of the curves furnished by this instrument, can be appreciated from the fact that, whereas neither the flour giving the top right-hand curve nor that giving the bottom curve is at all suitable for breadmaking, a fifty - fifty mixture of the two would give a well balanced and completely satisfactory dough, corresponding to a curve very similar in general shape to that of the satisfactory top left-hand curve.

The strength and stability figures furnished by various wheat samples when tested on the Alveographe, expressed in empirical units, are shown in Table I.

TABLE I

ALVEOGRAPHE FIGURES FURNISHED BY VARIOUS WHEATS

Type	Stability	General strength
Manitoba	80–110	65–105
Garnet	100–125	60–75
Canadian Durum	65–90	25–40
Hard Winter	60–90	40–70
Russian	50–85	30–65
Rosafe Plate	60–80	30–50
Barusso Plate	65–95	55–70
Australian	25–55	15–35
Indian	70–95	25–35
Danube Basin Wheats	30–65	25–40
Soft White Pacific	25–45	15–25
English	25–55	10–25

The other dough testing instrument in use in this country in which an applied stretching force and the degree of extension are measured is the Extensograph. In this instrument, shown in Fig. 9, a cylinder of dough supported at each end has a force applied mechanically to the middle so that it is stretched downwards into the form of an elongated U until it breaks. The applied force and the degree of extension are recorded on a moving band of ruled paper. As in the Farinograph, the doughs are tested at constant consistency and hence carry different amounts of water. The curves produced by the Extensograph are illustrated in Fig. 10. It is usual to attach importance to the maximum height of the curve, which is termed resistance, the width of the curve, which represents the extensibility, and the area enclosed by the curve, which is referred to as the energy and is a measure of the strength. The ratio of maximum height to width is used as an index of balance of properties; a low ratio indicates a soft "flowy" dough and a high ratio a stiff and tough dough.

Each of the three instruments that have been discussed, namely, the Farinograph, the Alveographe and the Extensograph, is being used for routine testing in cereal laboratories in this country and certainly permits various aspects of the baking qualities of flour and/or

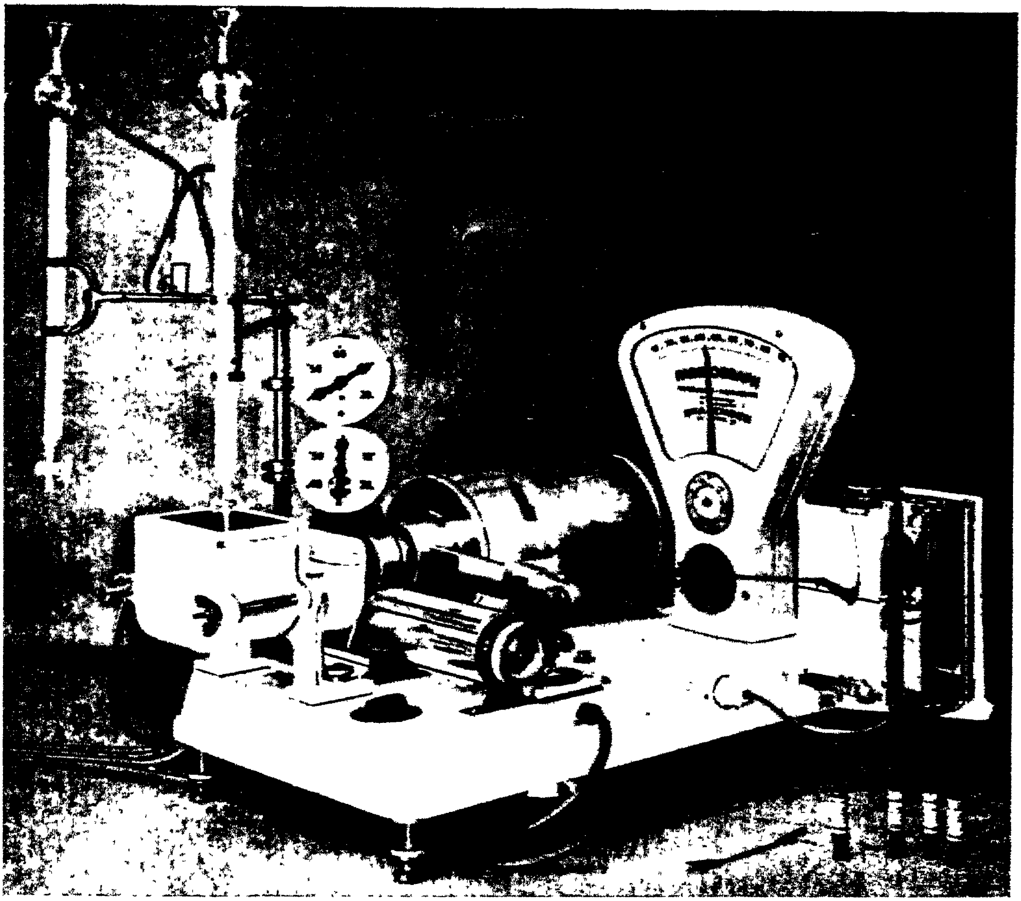


Fig. 1. The Brabender Farinograph

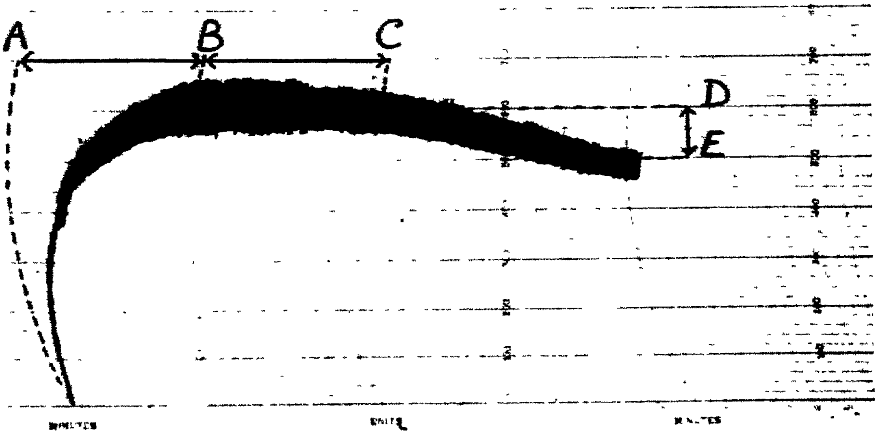
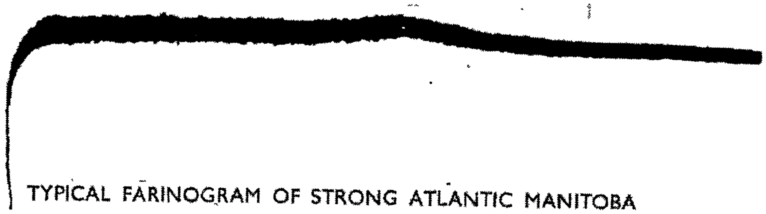
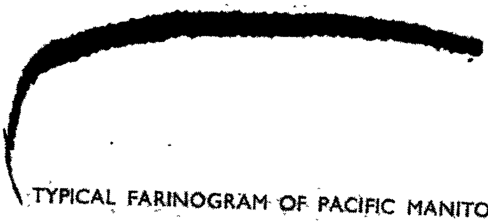


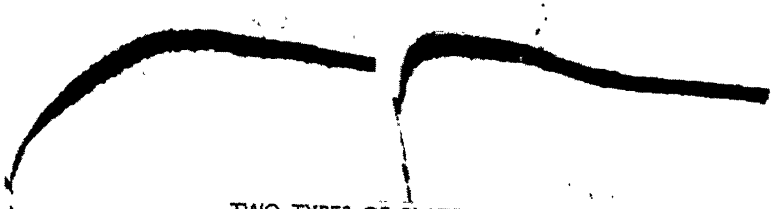
Fig. 2. A Farinograph curve



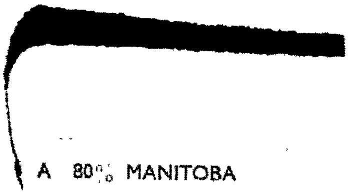
TYPICAL FARINOGRAM OF STRONG ATLANTIC MANITOBA



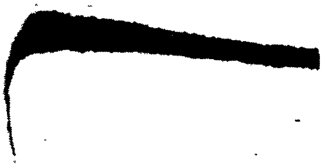
TYPICAL FARINOGRAM OF PACIFIC MANITOBA



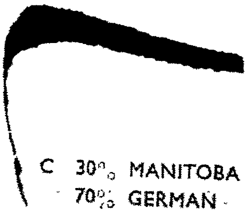
TWO TYPES OF PLATE FARINOGRAMS



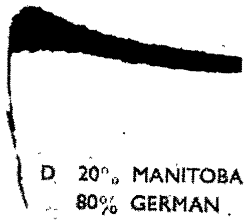
A 80% MANITOBA
20% GERMAN



B 50% MANITOBA
50% GERMAN



C 30% MANITOBA
70% GERMAN



D 20% MANITOBA
80% GERMAN

Fig. 3. Farinographs from strong and weak flours (Kent-Jones and Amos[4])

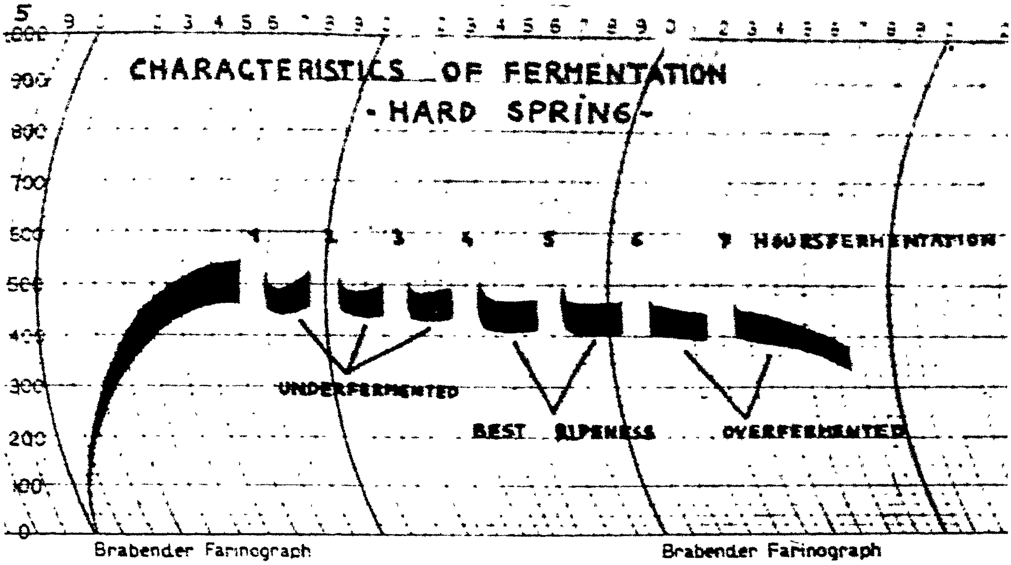


Fig. 4. Farinograph of Fermenting dough (Brabender[1])

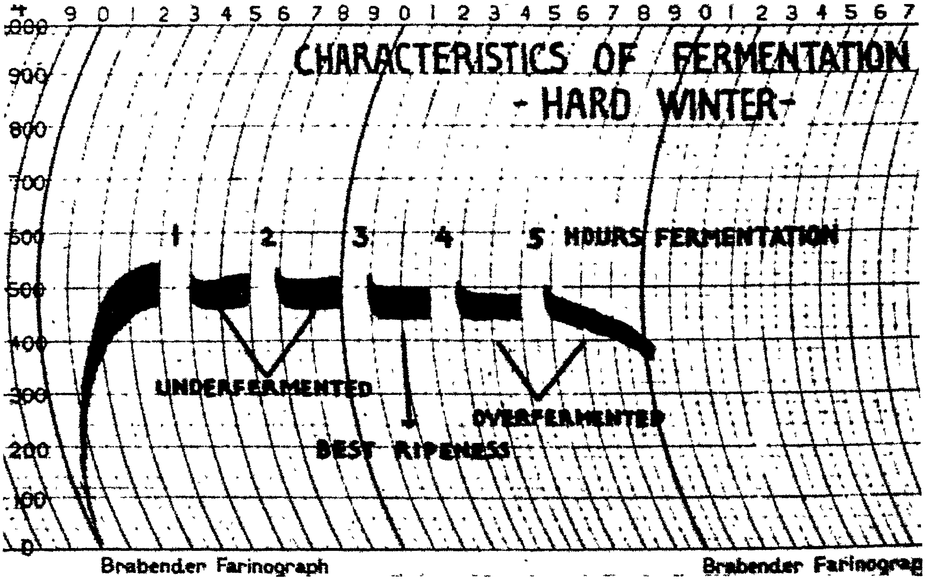


Fig. 4a. Farinograph of fermenting dough (Brabender[1])

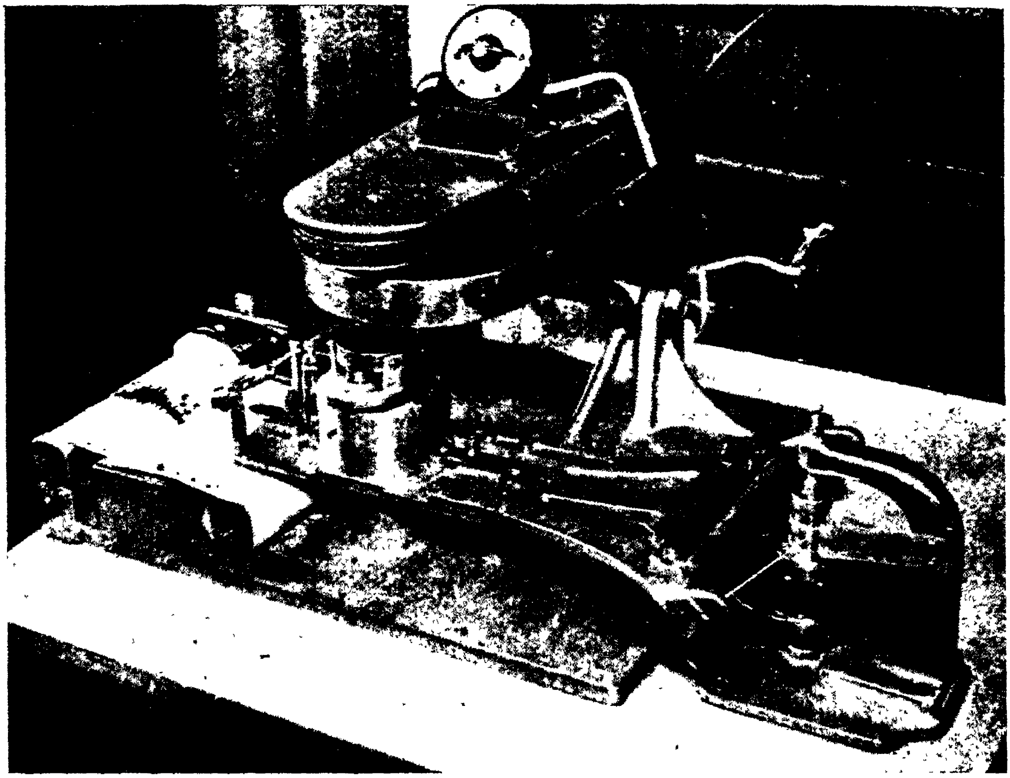


Fig. 5. The Mixograph (Kent-Jones and Amos[4])

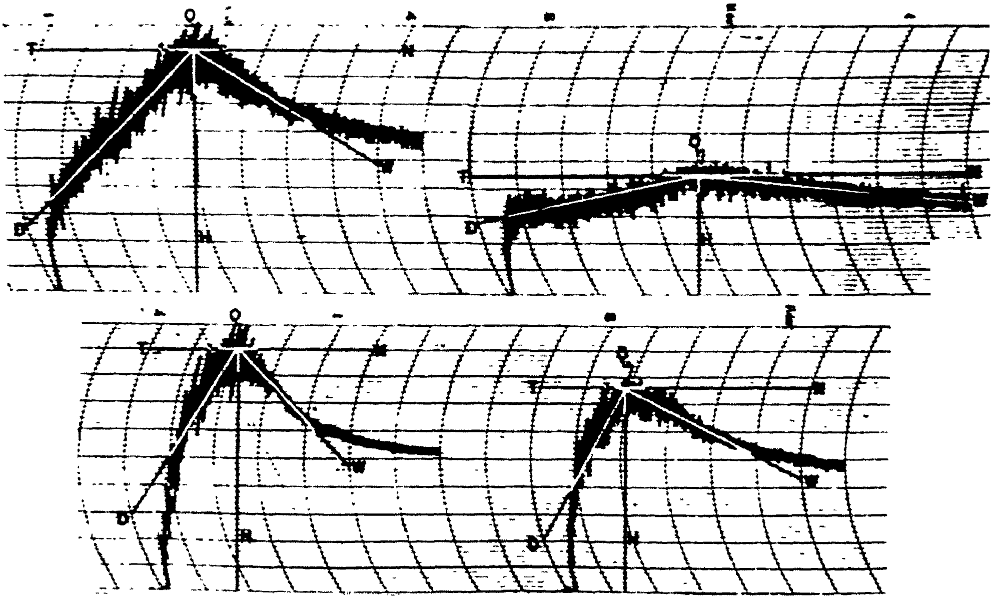


Fig. 6. Mixograph curves (Swanson and Johnson[9])

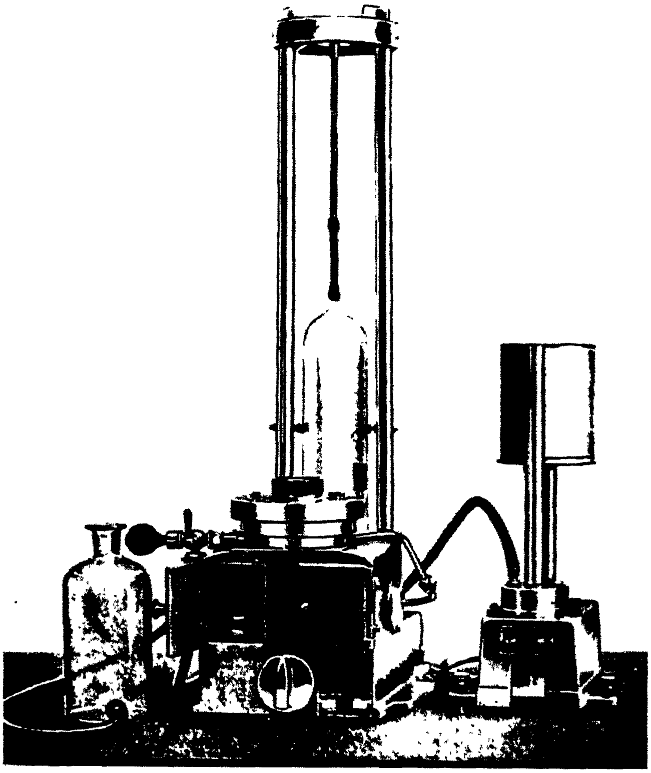


Fig. 7. Chopin Alveographe

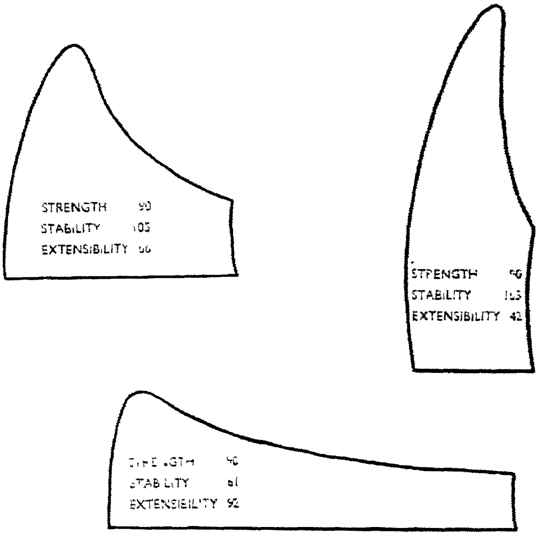


Fig. 8 Alveographe curves of equal area.

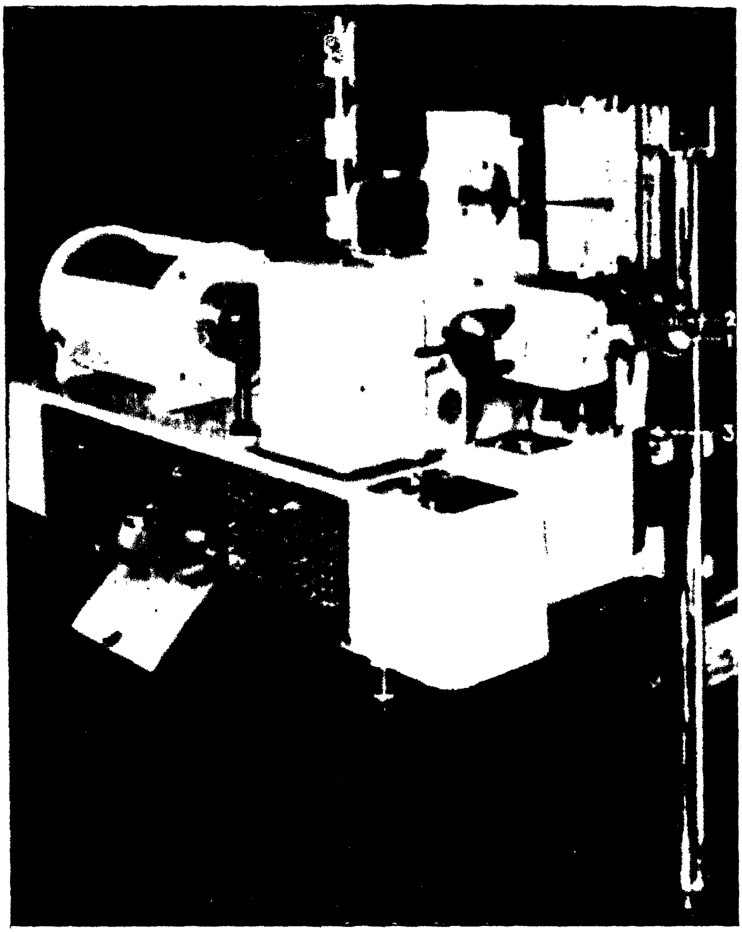


Fig. 9. The Brabender Extensograph

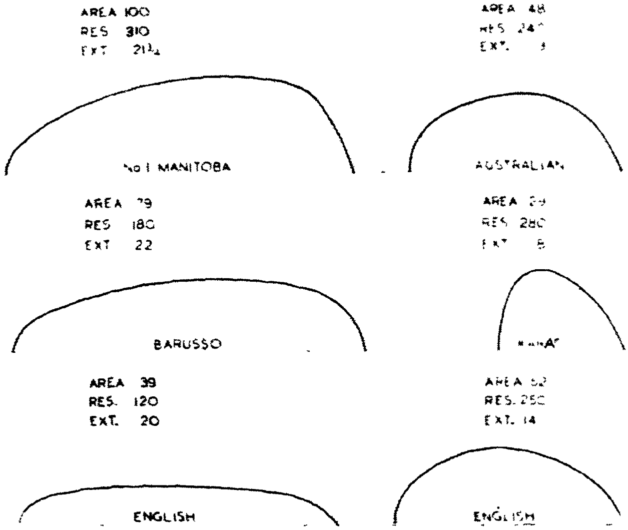


Fig. 10 Extensograph curves (Lockwood[5])

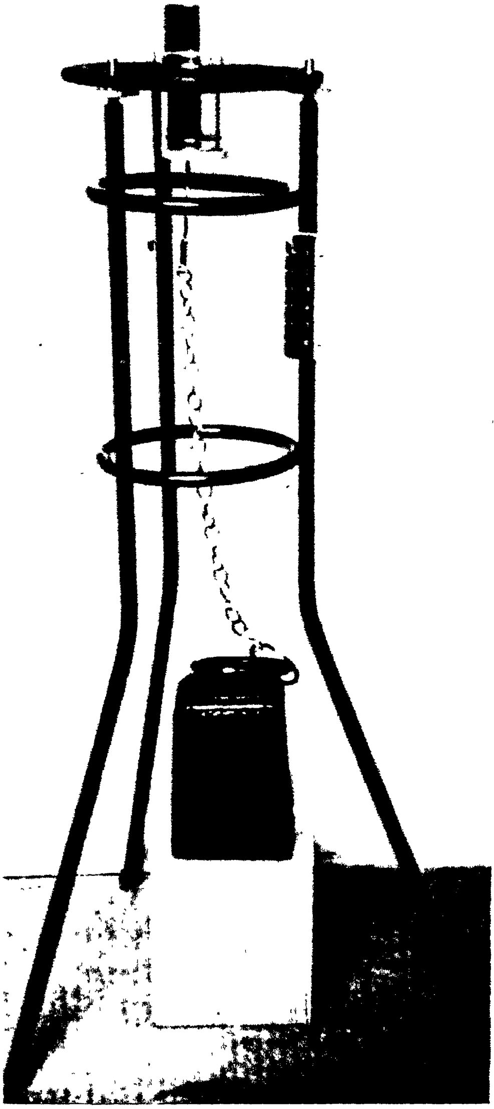


Fig. 11. Halton's Absorption meter

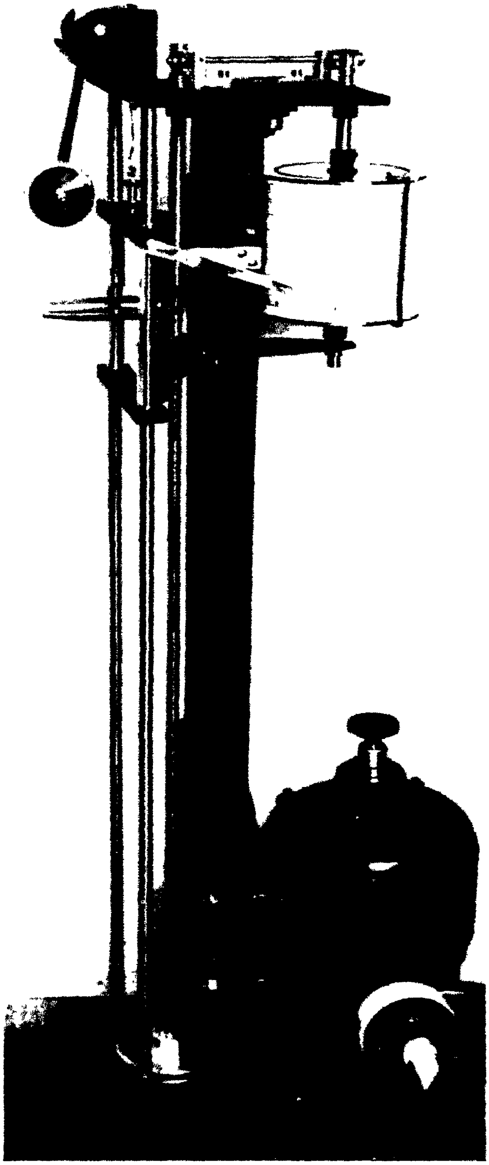


Fig. 12. Halton's Extensomete

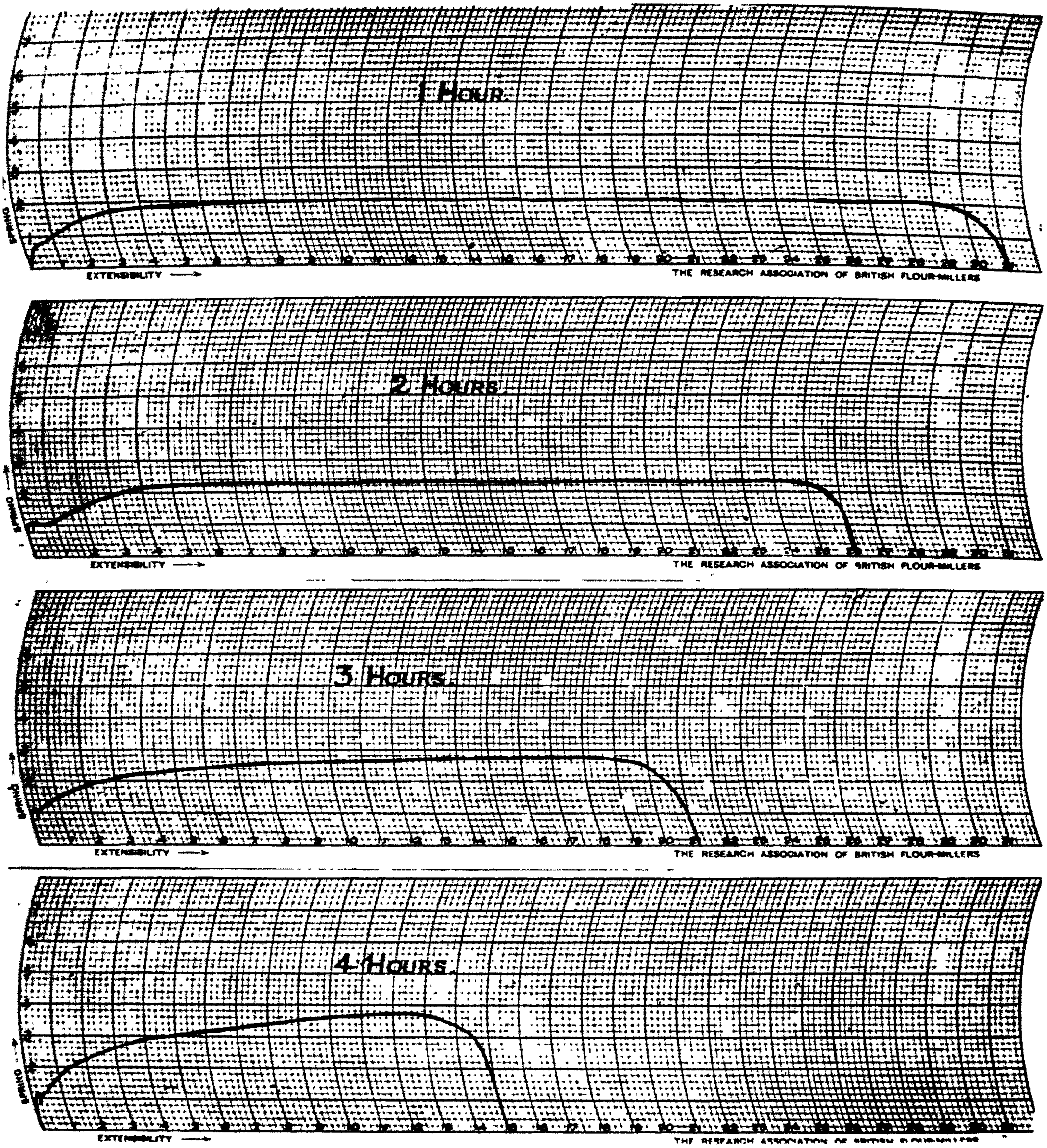


Fig. 13. Extensometer curves (Halton[2])

of the blending potentialities of wheats to be reliably assessed. Not one of these undoubtedly valuable instruments, however, is the outcome of study of dough properties from the angle of fundamental concepts; in each instance the sequence of events was the perfection of a method of recording the response of a dough to an imposed stress and the interpretation of the records in terms of baking behaviour determined in the test bakery. Schofield and Scott Blair,[6,7,8] however, made a fundamental approach to the problem by devising methods for determining certain strictly definable physical properties of flour dough, and this work was developed by Halton and Scott Blair,[3] who studied the relationship between these properties and baking quality. Extensive use was made by these workers of an apparatus in which a cylinder of dough floating on a bath of mercury is stretched by means of a given force for a given time and measurements are made of the extent to which the dough stretches and the extent to which it recovers after removal of the stretching force.

Halton and Scott Blair established that two of the most important factors in dough behaviour were viscosity and elastic modulus, two properties which they were able to measure in absolute units on their apparatus. They suggested that for the production of good bread a dough should possess high viscosity, since the viscosity is a measure of the stability of the dough, that is, its ability to resist flowing out under its own weight, but a low modulus, which would permit the dough to expand properly during fermentation and the early stages of baking. On this theory, good breadmaking value calls for a high viscosity/modulus ratio, a ratio which Maxwell termed "relaxation time." Halton and Scott Blair believe that what the baker terms the "spring" of a dough, *i.e.*, the degree to which a dough recovers after deformation, is closely associated with relaxation time. Since both the viscosity and the modulus of a dough are related to the proportion of water present, and the influence of water content is not the same for the two properties, the *ratio* of viscosity to modulus, or the relaxation time, for a dough will depend upon the proportion of water used in making the dough. Halton and Scott Blair decided, therefore, that they must bring each of their doughs to a water content which corresponded to optimum handling and baking quality for the particular flour used, or in other words, to the correct commercial water absorption.

Further investigation revealed that the amount of water a baker incorporated in his dough was determined not by the viscosity but by the modulus of the dough. In other words, doughs prepared by a baker for breadmaking from different flours would be likely to exhibit a wide range of viscosity, but their elastic moduli would be very similar. Halton and Scott Blair, therefore, decided to make their measurements of the relaxation time at a constant modulus which would correspond to the mean of the moduli of the doughs used in a range of commercial bakeries.

Following on from these fundamental studies, Halton has devised an instrument for determining the correct amount of water to be used in making the test dough and another instrument, a marked advance upon the original mercury-bath apparatus, by means of which the dough can be stretched mechanically and the stress/strain relationships automatically recorded. These instruments, however, are not yet on the market. His water absorption apparatus is shown in Fig. 11. A yeasted dough is prepared under standard and controlled conditions and after a 3-hour fermentation period is placed in the cylinder. It is then forced through an orifice in the bottom of the cylinder by a plunger operated by the attachment of a weight, and the time taken for a given volume of dough, as measured by the pointer, to be extruded is noted. It has been established that the extrusion time is a function of the modulus of the dough. The test is performed at three different levels of water content and a curve of extrusion time against water content is plotted. From this curve is read the proportion of water required to give the extrusion time which is known to correspond to the desired modulus.

Halton's dough testing instrument is shown in Fig. 12. A ball of yeasted dough prepared under standard and controlled conditions is pushed on to the two pegs until they have passed right through the middle of the ball. By means of the motor the lower of the two pegs is caused to descend at a constant rate, which stretches the dough and thereby exerts a force upon the upper peg. This peg is connected to a balance weight so that the force exerted on the peg causes it to move through only a small distance. The movement of this peg is recorded upon a moving band of ruled paper. The height of the curve drawn on the paper is a measure of the tension in the stretched dough, and the length of the curve is an index of the extent to which the dough is stretched.

Some curves produced on this instrument, which is called an Extensometer, are shown in Fig. 13. The product of the maximum height of the curve and the length of the curve, that is, the product of load and extensibility, is accepted as a measure of the potential strength of the dough. Since all doughs are made to the same modulus, the height of the curve is an index of the relaxation time of the dough, *i.e.*, of the viscosity/modulus ratio, and is, therefore, a measure of the spring of the dough. The length of the curve is, of course, a measure of its extensibility. The Extensometer curves in Fig. 13 were made at hourly intervals on the same dough and reveal the changes in the physical properties of a dough which occur as fermentation proceeds. In the early stages of the fermentation the dough is very distensible but has only fair spring, as is revealed by the 1-hour curve. As fermentation proceeds the dough becomes less distensible or "flowy," tightens up and becomes more springy, as can be seen from the gradual change in the shapes of the curves.

In Table II are given the results of Extensometer tests on doughs containing various quantities of chemical improvers, that is, substances that alter the physical properties of the dough in the direction of improved breadmaking quality.

TABLE II

EFFECT OF ADDITION OF IMPROVERS TO FLOUR ON EXTENSOMETER CURVES
(Halton, 1949)

	Height	Length	Product
Flour A:			
Potassium bromate, 0 p.p.m.	2·0	19	38
„ „ 10 „	2·4	16	38
„ „ 20 „	2·8	14	39
„ „ 30 „	3·2	12	38
Flour B:			
Nitrogen trichloride, 0 g./280 lb. . .	1·5	21	31
„ „ 3 „ . .	2·0	16	32
„ „ 6 „ . .	2·2	14	31
„ „ 9 „ . .	2·3	13·5	31

It will be seen that as the proportion of improver is increased the height of the curve becomes greater, which indicates more spring in the dough, whilst the length of the curve is decreased, indicating a reduction in the extensibility. An interesting feature is that the product of these two figures, which Halton calls the potential strength of the dough, is more or less constant. In other words, the action of an improver as judged by this instrument is not a matter of increasing the strength of the dough but rather the production of an optimum balance between the individual physical properties, so that the potential strength of the dough is fully realised.

This necessarily extremely brief survey of a very important and very complex subject can do little more than reveal the value of the information which the cereal chemist obtains as a routine measure by the application of rheological methods to doughs, and suggest problems that still await elucidation. It is true that the mechanical dough-testing instruments in regular use are empirical in principle and do not measure individual physical constants, but they nevertheless furnish information of very great value. There is little doubt, however, that the fundamental work which has been done in this field in recent years will be followed up and we may look forward to the appearance of new or improved dough testing instruments and to the disappearance of some of the empiricism of long-established dough testing methods in favour of an interpretation of results on a more fundamental basis.

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4. Kent-Jones, D. W., and Amos, A. J., *Modern Cereal Chemistry*, 4th Edition, 1947, Northern Publishing Co., Liverpool.
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88 MADELEY ROAD
EALING, LONDON, W.5

The Use of Rheological Tests in the Pharmaceutical and Cosmetical Industries

By ROBERT H. MARRIOTT

Many pharmaceutical and most cosmetical products are required to possess a "consumer appeal." The chemist employed in this branch of industry has therefore to pay attention to uniformity of production and the maintenance of a good appearance of the products, and also to consider fully the form of a product most likely to ensure that the user can employ it to the best advantage.

In order that a formulator can investigate the effect of modifications of a recipe and of the method of manufacture, and the production department can ensure a uniform product, there is need of a means of evaluating the consistency or "body" of a product. This is a complex summation of a variety of physical attributes and even if it were possible to resolve consistency into all its component items, the task of finding an equation in which each component contributed its correct quantum would be almost insuperable. The usual method adopted is the practical one of touching with the finger or squeezing a tube, methods only of use when the assessor is gifted in that way and has experience. Attempts by means of mechanical devices to imitate these tests never seen to be successful. It has been found, however, that the rotating cup viscometer does give values that have some sort of relationship with quality and are reproducible not only on samples from the same batch but also on the same sample, provided that the conditions of experiment are not varied.

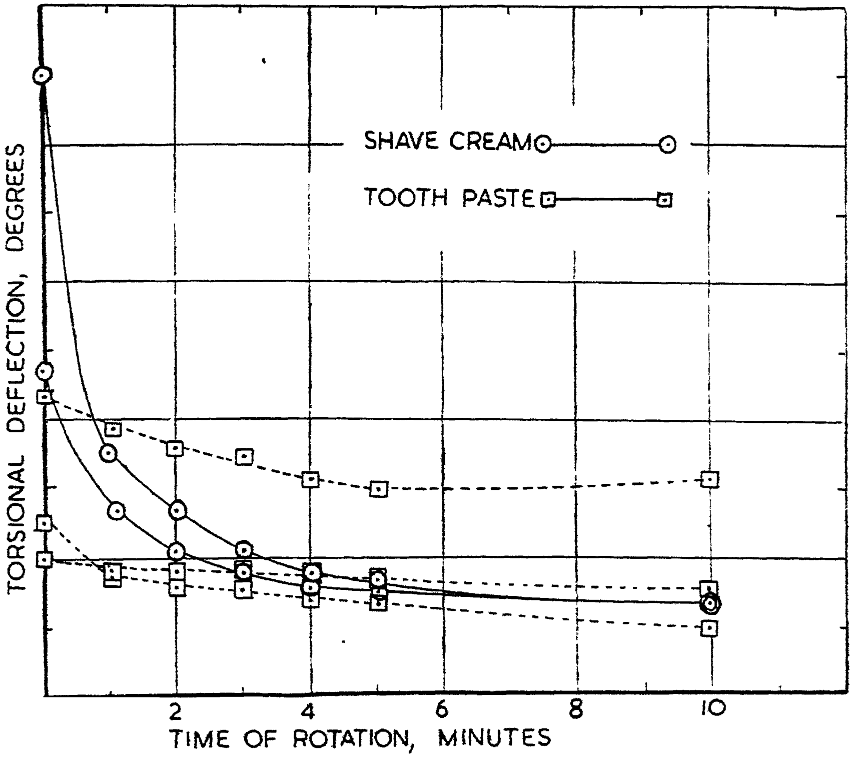

Fig. 1

It is the purpose of this note to indicate some of the ways in which such a viscometer can be applied in the works control laboratory. Mention is made of the value of this instrument in following the effect of variations in the manufacturing processes and of the changes that can occur in di- or multi-phase systems on storage.

Dentrifices and shaving and hair creams must possess "body." At the same time this body should not be so great that difficulty is experienced in the filling of the product into tubes or bottles. If the degree of "body" required of the preparation when in the hands of the user is such that filling would be difficult, then the cream or paste must be comparatively fluid when made and become firmer on storage, *i.e.*, it must possess thixotropy. When the cup of the viscometer is filled, the plunger dipped into the material under test and the motor started, the torque is taken up by the torsion wire until a point is reached when the plunger ceases to be carried round. The angular deflection at this point is a measure of the viscosity of the material. If the substance has structure, the torsional deflection will decrease as this structure is broken down and in the course of time the torsional deflection will reach a constant value. Fig. 1 shows curves for the torsional deflection plotted against time, for shaving cream and tooth paste. The upper of the two curves for shaving cream indicates the highest values found in practice for a particular well-known product and the lower curve gives the minimum permissible values if the product is to be deemed satisfactory. For the tooth paste, the highest curve is one relating to a popular brand and the two lower curves are characteristic of another make. These curves were obtained with the freshly made unstored product.

The effect of the conditions of manufacture of hair cream is indicated in Fig. 2. When the ingredients are mixed at a slow rate of stirring, the cream is almost Newtonian in character. As the rate of stirring is increased, the amount of thixotropy becomes more marked and at the same time the viscosity becomes significantly greater. The top curve indicates the values for the emulsion when made in large bulk, but prior to homogenisation.

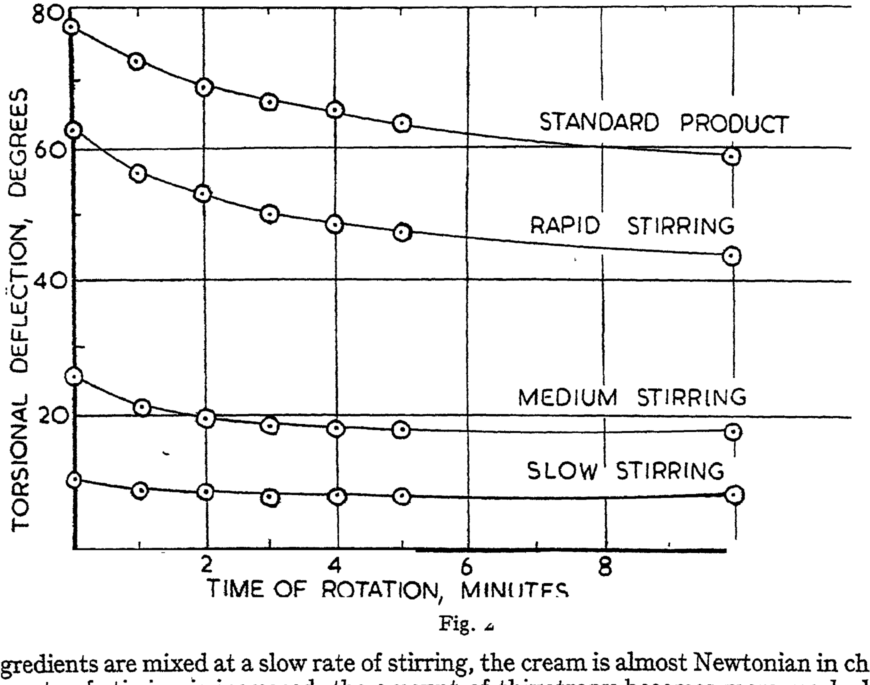

Fig. 2

In making ointments or lotions, cetyl alcohol is often employed as a stabiliser of the emulsion. While it is known qualitatively that increasing the percentage of the fatty alcohol increases the body or consistency, the rotating cup viscometer enables a numerical assessment of this effect to be rapidly made. Fig. 3 shows the torsional deflection plotted against the cetyl alcohol content of an oil-in-water emulsion. The upper line shows the variation in the yield value, the lower the variation in the equilibrium viscosity. The distance apart of the two curves is a measure of the thixotropy.

Fig. 4 shows the equilibrium viscosity values of shaving cream, tooth pastes and hair cream plotted against time of storage at ordinary laboratory temperature. The shaving cream appears to reach a constant value after about 8 weeks. One of the tooth pastes rapidly increases in viscosity, probably attaining an equilibrium in 2 weeks, whilst the other appears to require about 12 weeks. The hair cream behaves rather differently in that there is only a slow increment for the first 4 weeks, after which the consistency rises rapidly to a constant value. During the increase in the equilibrium consistency there is also a gain in the yield value, the thixotropy, *i.e.*, the difference between the yield and the equilibrium values, often becoming greater. In this state the cream would be difficult to shake out of the bottle and it would be necessary to stir it or else subject it to vigorous shaking.

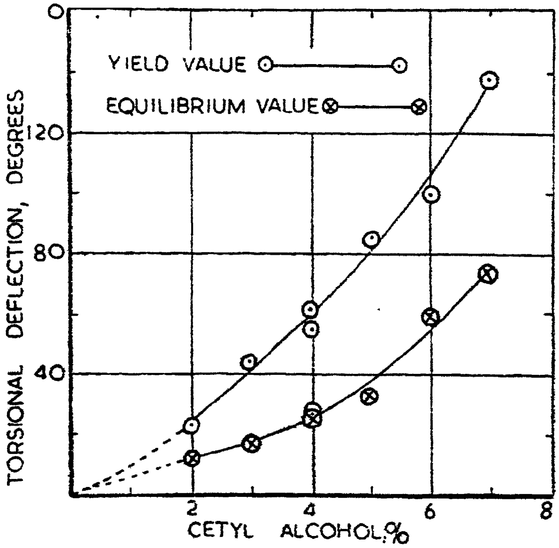

Fig. 3

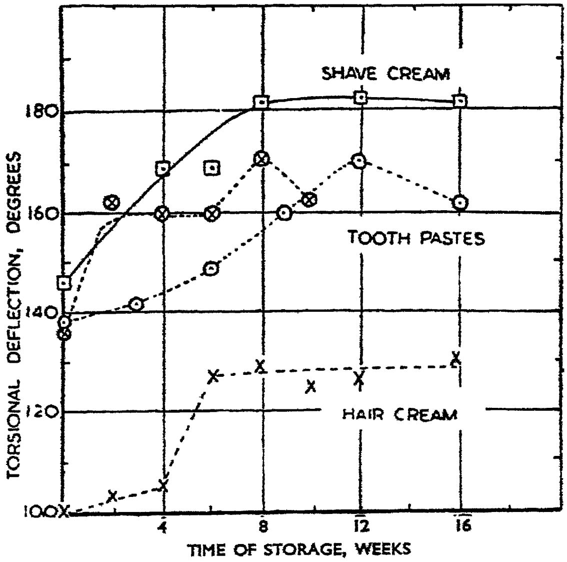

Fig. 4

It will be seen that this form of viscometer can be used to give a numerical assessment value to pastes and creams which will enable the manufacturer to maintain a constant quality. Its value in the factory is twofold. Firstly, the determination can be quickly carried out, so that the rate of production is not prejudiced, and secondly, it enables a mass of experience to be built up which will lead to a quick decision as to the steps to be taken to rectify a mistake and to prevent any subsequent repetition.

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The Application of Rheological Methods to the Paint Industry

By P. S. WILLIAMS

I shall describe briefly the processes involved in paint manufacture and the desirable rheological properties of a paint, before discussing some of the control viscometers and research methods used in the paint industry. In preference to dealing in detail with some small aspect of paint rheology, I propose to give a general impression of apparatus and methods used to study the flow characteristics of paints and allied materials.

Paints consist of pigments dispersed in liquid media. The pigments used may be inorganic or organic, and of a wide variety of shapes and sizes. Fig. 1 shows four typical paint pigments, varying in shape from acicular crystals to amorphous powder, and in size from a fraction of a micron to several microns. The medium may be a drying oil, a varnish or a nitrocellulose solution. In order to disperse the pigment in the medium, some mechanical action is necessary, and to assist in this process of dispersion—called milling—small quantities of dispersing or wetting agents are sometimes added to the paint. The milling is often carried out with only a portion of the total quantity of medium and solvent finally to be used in the paint, so that further quantities of liquid have to be added, after milling, to bring the paint to a suitable application consistency. It is this bringing of a paint to a consistency suitable for application that calls for control viscosity measurements, and it is the rather unpredictable flow behaviour of pigment suspensions in general that calls for research.

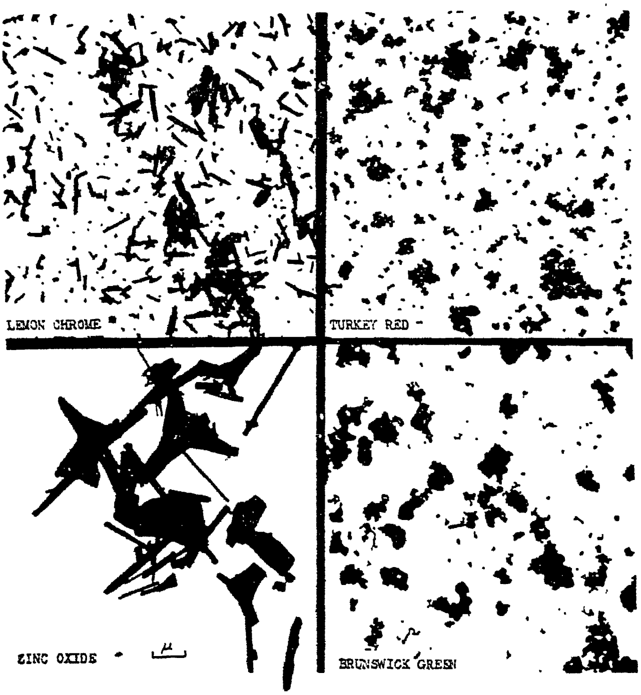

Fig. 1

There are three main methods of applying paint to a surface: by brushing, spraying or dipping, and the requirements of a paint for application by each of these methods are not the same.

Brushing—It is necessary that the paint shall work well under the brush, that is, there shall be little drag or pull on the brush, otherwise the painter will be tired after a short time. In addition, the paint must have sufficient "flow," so that after the brush has left the surface, the brush-marks will flow out and a smooth finish result. On the other hand, this flow must be so controlled that no sags, or tears, appear on the finished article.

Spraying—For this it is necessary for the paint to contain more solvent than is present in the brushing material. The consistency must be such that the paint can be atomised by the spray-gun, and sufficient solvent must be present to allow for loss by evaporation during transfer from the gun to the surface being painted. The film so produced must have sufficient "flow" to prevent what is known as "orange-peeling," the name given to the condition that arises when the droplets of paint, impinging on the surface being coated, remain in position instead of flowing out to give a smooth finish. On the other hand, as with brushing paints, the flow must be controlled to prevent sagging.

Dipping—In this method, as the name suggests, the articles are completely immersed in the paint, and then removed at a controlled rate. Here, the important factor is to control the flow so that films of the right thickness are produced.

It is clear that whether a paint is to be applied by brushing, spraying or dipping, its rheological properties, or more simply its viscosity, is a factor of prime importance. Unfortunately, the viscosity of a paint is not a true physical constant but depends on many factors. The types of rheological behaviour encountered in the paint industry may be classified under the main headings of Newtonian, thixotropic and dilatant. Fig. 2 shows the general shape of curves obtained by plotting stress against rate of strain for these types of flow. Of particular importance is the study of the flow behaviour represented by the thixotropic curve. Paints are subjected to a high rate of shear during brushing or spraying and may then appear to flow readily, yet the rate of flow under such small forces as surface tension and gravity may be very small indeed and, in fact, flow may cease altogether, resulting in brush-marks or other defects. Another complication from the rheological testing point of view arises from the time dependence of the viscosity of a paint. By time dependence, I mean that a paint, at rest in a container, will have a certain apparent viscosity, but that on stirring the paint, the viscosity will decrease and regain its former value on leaving at rest for some time, maybe hours, maybe minutes. These two factors, the dependence of flow on applied shear stress and on time, are generally grouped together under the term thixotropy. A certain degree of thixotropy is desirable in a paint, for it is the "physical" increase in viscosity that holds a paint in position against gravity forces, before the chemical drying has begun to take effect.

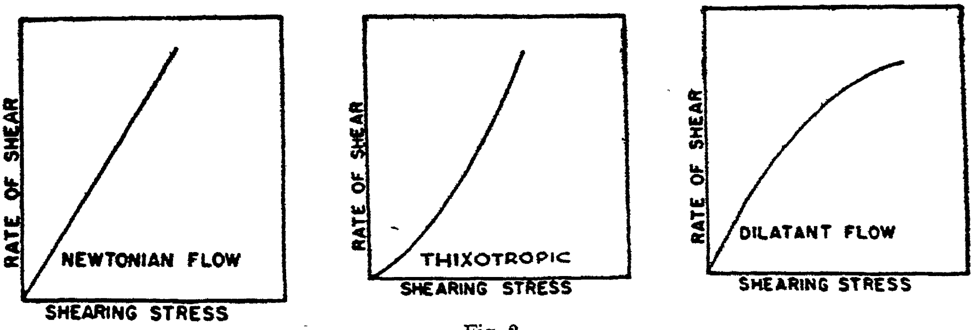

Fig. 2

Many materials exhibit thixotropy and several mechanisms have been postulated to account for the phenomenon.[1,2,3,4,5] One suggests that the material is a loose network of particles interconnected at a few points, the network enclosing and immobilising dispersion medium. A second picture is that particles do not form any connected structure but are set and held in the dispersion medium by long-range forces. It is most likely that a combination of mechanisms applies to most systems, but further work must be done before any satisfactory theory can be formed.

I will now describe some of the instruments and experimental methods used to determine viscosity and to assess thixotropy. Efflux viscometers are most widely used by the industry for control purposes. There are very many types in use—a few of them standardised—and all of them are used in the following manner. The cup is filled with the paint to be tested and the time taken for all, or some specified volume, to flow through the orifice is a measurement of the viscosity. Although these cup viscometers are quite suitable for use with

Newtonian liquids such as most oils, varnishes and lacquers, they have serious limitations when some paints are tested in them, for often near the end of efflux there is not sufficient hydrostatic head to overcome the structure in the paint, and even if the time of efflux of the first 50 ml. is taken, there is often wide variation due to breakdown of thixotropic structure in filling the cup.

Another type of viscometer enjoying some popularity abroad, as a control instrument, is the Gardner Mobilometer. This consists of a cylinder containing the material under test through which a plunger is forced. The plunger consists of a metal rod passing through a guide, and on its end is screwed a disc, perforated with holes. Loads are applied to the plunger, and velocity of fall against load is plotted. A very wide range of materials can be studied using various shaped discs provided, and some qualitative estimate of degree of thixotropy can be formed by stirring with the plunger, allowing structure to reform for some set period and then making the measurement.

Turning now to the field of varnish and bodied oil manufacture, we have viscosity measurement as the control deciding how long to heat the ingredients to give the required degree of polymerisation. In making an oleo-resinous varnish, a resin and an oil are heated together for a certain time. The molecular weight of the varnish increases as the cooking proceeds, and the viscosity is measured at frequent intervals so that heating may be stopped when the material has reached the required degree of polymerisation. It is unfortunate that at the elevated temperatures used in varnish making, the differences in molecular weight have little effect on the viscosity, although at room temperature they cause large differences in viscosity. The usual method of viscosity measurement for varnishes is to test a sample by means of "body" or "bubble" tubes. These are tubes of standard size, about 10 cm. long and 1 cm. in diameter, containing mineral oils of known viscosity. A sample of liquid to be tested is put in a similar tube, leaving a bubble about the same size as that in the standard tubes. By comparing the times of rise of bubbles, the viscosity of the material may be readily estimated. This method is quick and requires only a small sample—an important point in varnish manufacture—for until the viscosity determination has been made, the material is undergoing further polymerisation and there is risk of spoiling a batch.

A method for determining the viscosity of a paint or varnish in a large container and not requiring the withdrawal of a sample would be of considerable value. The Brookfield viscometer is used for this purpose in America. This viscometer uses a synchronous motor to drive a shaft at a constant speed, one of four speeds selected by a gear box. The shaft is coupled by a torque-measuring spring to a cylinder which is dipped into the liquid under test. The drag on this cylinder is used to give a dial reading in centipoises, and by using cylinders of different sizes a wide range of viscosities can be covered.

For accurate viscosity measurements there are the well-known methods using B.S.I. U-tube viscometers or B.S.I. falling sphere viscometers. To find the viscosity of a small quantity of very viscous liquid such as a drying oil or varnish, an extension of the B.S.I. falling sphere method is very useful, and rapid. The liquid is contained in a body tube and the B.S.I. technique is followed, except that the ratio of ball to tube diameter, d/D, is greater than 0·1. Owing to the close proximity of the wall of the containing vessel, care must be taken to apply the correct wall-correction factor which Faxen[6] has derived, and which is given by the bracketed term in the expression

$$\eta = \frac{2\,gr^2}{9\,v}(\sigma - \rho)\left[1 - 2{\cdot}104\left(\frac{d}{\mathrm{D}}\right) + 2{\cdot}09\left(\frac{d}{\mathrm{D}}\right)^3 - 0{\cdot}95\left(\frac{d}{\mathrm{D}}\right)^5\right]$$

where d = diameter of sphere, radius r; D = diameter of cylindrical tube; v = velocity of sphere; and σ and ρ are the densities of the sphere and liquid, respectively.

I will now discuss recent research methods and their uses. Recent techniques seem to favour concentric cylinder apparatus so that the material being sheared between two cylinders may be subjected to known steady rates of shear for any duration. By measuring the torque on the inner cylinder and rotating the outer, or *vice versa*, it is possible to cover a wide range of shear stresses.

Green and Weltmann[7,8,9] in America have developed a technique for giving some quantitative measure to thixotropy. This they do by constructing and analysing hysteresis loops for their materials. Fig. 3 shows such a loop, which is obtained by plotting deflection of inner cylinder against speed of rotation of outer cylinder for increasing speeds. The down curve is obtained by quickly lowering the speed of rotation to zero. From analysis of this loop, the authors derive two coefficients of thixotropic breakdown; one is independent of time and may be defined as the loss in shearing force per unit area per unit increase in velocity gradient for the selected time employed in making the up curve. The other gives a relation between the total length of time employed in producing the breakdown, and the breakdown itself. Application of this technique has been made by Fischer and Jerome,[10] who studied the effect of milling conditions, wetting agents and variation of medium on flow properties of suspensions. Table I shows a few of the results they tabulate, and it is evident that the effect of the wetting agent is highly specific to the system being investigated.

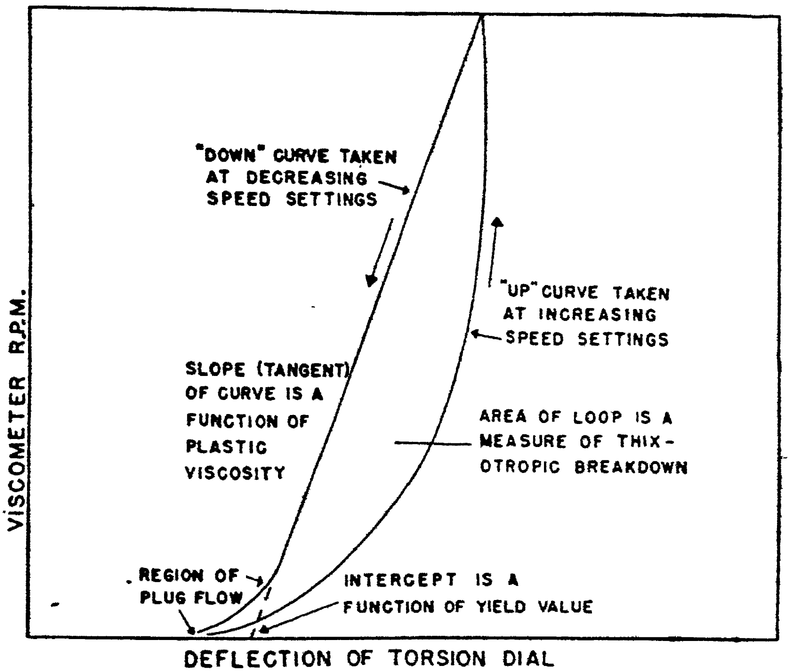

Fig. 3

TABLE I

EFFECT OF SURFACE ACTIVE AGENTS ON FLOW PROPERTIES

(Fischer and Jerome[10])

	Carbon black				Iron blue				Ultramarine blue			
	9·7% in glycerol		15·3% in stand oil		29·8% in glycerol		28% in stand oil		39·7% in glycerol		32% in mineral oil	
Reagent	V	*f*	V	*f*	V	*f*	V	*f*	V	*f*	V	*f*
None	3·0	3800	57	10,400	8·3	37	90	2600	41	0	46	1900
Benzidene ..	17·0	5870	53	770								
Tergitol 7 ..	4·4	820	65	10,700								
Sapamine K.W.					70	8000	81	2300				
Lecithin.. ..									65	7700	23	74
Zinc naphthenate									60	1970	35	180

V = plastic viscosity. *f* = yield value.

A new type of viscometer described by Wachholtz and Asbeck[11,12] has been used to study pigment - oil dispersions. Called a band viscometer, the instrument consists of two metal blocks, A (Fig. 4), which have a recess in the top, acting as a reservoir. The band C is drawn through the slit formed between the two faces of the blocks by thin metal spacers, B. The whole assembly is clamped together and held in a stand. At the lower end of the band, which is 60 cm. long, 3 cm. wide and 0·005 cm. thick, are hung weights, and the velocity of fall of band is plotted against load. Of particular interest is the method of plotting results. The authors use a value η_∞ which is the viscosity the material would have at infinitely large rate of shear. This value η_∞ is obtained by plotting apparent viscosity against $\sqrt{1/D}$, where D is the rate of shear, and extrapolating to $D = 0$. Relationships between viscosity and pigment concentration have been examined by using η_∞, since various anomalies present at low rates of shear disappear at the very high shear rates of the band viscometer, as indicated by the statement that the curve against pigment concentration suffers no change in shape when the suspending medium is changed from a mineral oil to a vegetable oil.

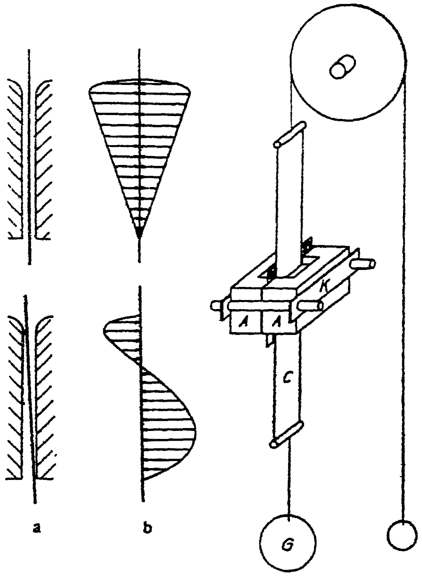

Fig. 4

Finally, I will mention alternating stress techniques. A long time ago it was realised that most paint systems are visco-elastic, and Pryce-Jones[13] made some qualitative experiments showing that the elastic component of many suspensions is quite large. Recently, techniques have been developed whereby materials instead of being subjected to a unidirectional shear stress may have alternating stresses imposed on them, or superimposed on a unidirectionally applied stress. Weissenberg[14] in England, and Sandvik and Goldberg[15] and W. P. Mason[16] in America, have developed apparatus for determining modulus of rigidity and dynamic viscosity of visco-elastic materials. Nearly all previous methods demand that the sample undergoing test suffers large deformations. By decreasing the amplitude of oscillation, in alternating stress methods, it is possible to study the rheological behaviour of a material without disturbing its state.

I have indicated some of the control methods used in the paint industry and their limitations, and have outlined briefly research methods for studying paint systems. It is likely that in the future we shall see a wider use of such methods in the control of manufacture, for it is becoming increasingly important to standardise our products more rigorously.

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DISCUSSION ON THE FOUR PAPERS ABOVE

Mr. S. W. BUTTERWORTH asked Mr. Williams if alternating stresses at supersonic frequencies had been used in assessing the rheological properties of materials.

Mr. WILLIAMS replied that the maximum frequency used in mechanical alternating stress techniques was about 10,000 cycles per minute. The work of W. P. Mason, using torsionally oscillating crystals, covered the range 0 to 100 kilocycles per second, and so *did* use vibrations in the supersonic region. Although the shear waves set up in the liquid were too highly attenuated to be studied, they introduced a loading on the crystal which altered the resonance resistance and frequency of the crystal. From these values the viscosity and shear stiffness of materials might be calculated.

Mr. SYDNEY C. BEARD asked Dr. Marriott if the yield value depended on the time that elapsed while the sample was in the machine before the test began and whether a standard time had been found necessary. With cetyl alcohol emulsions, did the yield value and the viscosity depend on the degree of dispersion of the cetyl alcohol in water?

Dr. MARRIOTT said that the yield value did depend on the time that elapsed before the test was begun, but in practice it was not found necessary to take any special precautions. If it were required to determine the yield value, then a standard time must elapse before the machine was started. Regarding the effect of the degree of dispersion of the cetyl alcohol on the yield value and viscosity of emulsion made with it, the experimental figures shown were derived from emulsions made up under standard controlled conditions and they were therefore comparable with one another. The degree of dispersion of the cetyl alcohol was not known.

Mr. J. F. WOOD asked Dr. Marriott what standards were used to calibrate the revolving cup viscometer and in particular whether he could detect drifts in the characteristics of the instrument with continued use.

Dr. MARRIOTT replied that in the instrument described no calibration was employed, but it would be quite possible to use a suitable oil as a means of checking it. A number of viscometers of the design shown had been made and they were found to give similar readings when the same material was examined.

Mr. A. GELLMAN asked if Mr. Williams had anything to say about the methods of measuring the consistency of some of the heavier products of the paint industry, such as pastes and putties. Did he think some of the instruments described by other speakers might be useful for this purpose.

Mr. WILLIAMS suggested that the Institute of Petroleum testing cone-resistance penetrometer and the instruments described by Dr. Amos would deal with pastes and putties. Of the instruments he himself had mentioned, the Gardner Mobilometer would be most suitable for measurements on these materials.

Dr. C. G. SUMNER, apropos of Mr. Gellman's question, drew attention to two recent papers dealing with materials of that kind: one on "The Rheology of Stiff Pastes" by A. G. Ward and P. R. Freeman (*J. Scientific Instr.*, 1948, 25, 387), and the other, "The Rheology of Building Mastics. I. Caulking Compounds for Gum Application" by A. G. Ward and E. L. E. Westbrook (*J. Soc. Chem. Ind.*, 1948, 67, 389).

Studies in the Analytical Chemistry of Tungsten

IV. Separation of Tungstic and Arsenic Acids

BY D. A. LAMBIE

THIS paper, which treats of the separation and determination of tungstic and arsenic acids, continues the study of the complex-forming acid radicals begun, with the separation of tungstic and phosphoric acids, in Section II of this series.[1]

It was anticipated by analogy with phosphorus that solutions containing both tungstates and arsenates would yield on acidification arsenotungstic acid, most probably the "12" acid. This would render the precipitation of tungstic acid by means of mineral acid incomplete unless an alkaloid, *e.g.*, cinchonine, were added. The tannin phenazone procedure on the other hand, in which the precipitant is added to the alkaline solution,[1,2] would be expected to give a quantitative recovery of tungstic acid free from arsenic.

Although the main object of the investigation was to test the applicability of the tannin-phenazone method[2] to the separation of tungstic and arsenic acids, a critical examination of other methods in general use was also undertaken, as only that of Dieckmann and Hilpert[3] appears to be supported by published test analyses.

Since tungsten-bearing materials are invariably obtained in solution by means of oxidising reagents, the investigation was limited to the behaviour of quinquevalent arsenic. Also, since in practice it is usual, for simplicity of "opening up," to determine arsenic and tungsten in separate portions of material, this convenient subdivision of treatment has been adopted in the present work.

A. SEPARATION AND DETERMINATION OF ARSENIC

There is no doubt that in absence of interfering elements the classical iodimetric titration is the most accurate method for the determination of a milligram or more of arsenic. Experiments to determine the effect of tungstic acid on this method showed that the presence of a few milligrams caused significantly low results (see (*a*) below) and confirmed that separation of arsenic prior to its determination is essential.

The separation of Group II metals from tungsten by precipitation as sulphides from tartaric acid solution has been studied extensively for one of them, molybdenum, and is advocated for arsenic in standard works such as those of Schoeller and Powell[4] and Hillebrand and Lundell.[5] An examination of this method has therefore been carried out, although there is little doubt that distillation as trichloride from a solution containing excess of phosphoric acid (to retain the tungstic acid in solution as a complex) as advocated by Dieckmann and Hilpert (*loc. cit.*), provides the most satisfactory method of separating arsenic from tungsten.

(*a*) EFFECT OF TUNGSTEN ON THE IODINE TITRATION OF ARSENITE—Two series of experiments were conducted. In the first, solutions containing sodium tungstate and arsenite were rendered feebly acid to litmus paper, treated with excess of sodium bicarbonate and titrated as usual with 0·1 *N* iodine solution. As can be seen from Table I, Experiments 1 to 5, low results were obtained.

In the second series solutions of arsenite and tungstate, volume 100 ml., were treated with 10 ml. of hydrochloric acid, saturated with hydrogen sulphide, boiled and re-treated with hydrogen sulphide. The precipitates were collected on a filter, washed, "fumed" with sulphuric acid and titrated with iodine solution as usual. The tungstic acid was recovered from the filtrate by the tannin phenazone procedure and the amount of tungstic acid co-precipitated with the arsenic found by difference. Low results for arsenic (Experiments 6 to 9) were obtained as in the first series, although there is no evident proportionality between tungstic acid content and the magnitude of the error.

TABLE I

Expt.	Tungstic oxide taken, g.	Arsenious oxide taken, g.	Arsenious oxide found, g.	Error g.	Tungstic oxide in titrated solution g.
1	0·0798	0·1237	0·1202	−0·0035	0·0798
2	0·0798	0·0495	0·0467	−0·0028	0·0798
3	0·0798	0·0247	0·0222	−0·0025	0·0798
4	0·0200	0·1237	0·1232	−0·0005	0·0200
5	0·0200	0·0247	0·0247	nil	0·0200
6	0·0798	0·1237	0·1174	−0·0063	0·0397
7	0·0200	0·1237	0·1222	−0·0015	0·0080
8	0·0200	0·0247	0·0237	−0·0010	0·0159
9	0·0399	0·0247	0·0242	−0·0005	0·0320

(*b*) SEPARATION OF ARSENIC BY PRECIPITATION AS SULPHIDE—Examination of the literature revealed two points requiring investigation: (i) Werz's assertion,[6] which appeared to be based on insufficient evidence, that for the formation of a stable tungsto-tartaric complex, the tungstate solution must remain or be rendered strongly alkaline after addition of the tartaric acid; (ii) the possibility that contamination of the sulphide precipitate with tungstic acid when it occurs is due to post-precipitation. An alternative explanation of Werz's results is that the final excess of mineral acid is greater when the solution is not initially strongly alkaline, and consequently leads to "tartaric hydrolysis."[7]

Stability of tungsto-tartaric complexes—To test the stability towards hydrochloric acid of tungsto-tartaric complexes produced in alkaline and in acid solution, two groups of solutions similar in content of tungstic and tartaric acids were prepared. Each solution of the first group was rendered alkaline by addition of 10 ml. of 30 per cent. sodium hydroxide solution, heated, treated with 35 ml. of concentrated hydrochloric acid, boiled for a few minutes and after addition of filter paper pulp allowed to settle overnight. Those of the second group were heated and treated with a mixture containing 10 ml. of 30 per cent. sodium hydroxide solution and 35 ml. of concentrated hydrochloric acid, boiled for a few minutes and allowed to stand after the addition of filter paper pulp as before. Each pair of solutions had the same final composition and volume, the only difference being that the first of each was strongly alkaline before addition of the hydrochloric acid. The precipitates were collected, ignited and weighed. The more stable the complex the smaller should be the weight of tungstic oxide recovered. As is seen from Table II the stability increased (recovery decreased) with increase in tartaric acid concentration, as was of course to be expected, but no support was given to Werz's hypothesis that formation of the complex in strongly alkaline solution increases its stability.

TABLE II

Tungstic oxide taken in each experiment, 0·0998 g.

Expt.	Tartaric acid added g.	Tungstic oxide recovered, g. Complex formed in alkaline solution	Complex formed in acid solution
10	1	0·0993	0·0981
11	2	0·0978	0·0993
12	3	0·0763	0·0855
13	4	0·0393	0·0253
14	5	0·0232	0·0192

Although precipitation of arsenic sulphide is most satisfactorily done from strongly acid solution, here the acidity had to be maintained as low as was compatible with the reduction of quinquevalent to tervalent arsenic, owing to the instability of the tungsto-tartaric complex in presence of excess of mineral acid. After preliminary tests the following conditions for the separation were adopted.

Procedure—Add to the almost neutral solution of tungstate and arsenate—volume about 200 ml.—tartaric acid (or its equivalent of sodium tartrate) 30 times the weight of tungstic oxide present, followed by 5 ml. of 10 per cent. potassium iodide solution and 5 ml. of diluted sulphuric acid (1 + 1). Heat to about 80° C. and pass a rapid stream of hydrogen sulphide. Repeat the heating and treatment with hydrogen sulphide until the arsenic sulphide flocculates and settles rapidly, leaving a clear colourless supernatant liquid. Collect the precipitate and wash thoroughly with 1 per cent. v/v sulphuric acid containing hydrogen sulphide. Test the filtrate for completeness of precipitation by boiling and treating with hydrogen sulphide. Return the precipitate completely to the precipitation flask, boil to fuming with sulphuric acid and sodium sulphate[8] and complete as usual iodimetrically, or if the estimated amount is less than 1 mg., use the molybdenum blue colorimetric method.[9]

In a series of experiments, the results of which are given in Table III, the solution after titration was analysed for tungsten by the tannin phenazone method (see B (*b*)).

TABLE III

Expt.	Tungstic oxide taken, g.	Arsenic oxide taken, g.	Arsenic oxide found, g.	Error, g.	Tungstic oxide in arsenic solution, mg.	Tartaric acid added, g.	pH of solution
15	0·10	0·0994	0·0995	+0·0001	not detd.	3	1·0
16	0·10	0·0994	0·0995	+0·0001	0·05	3	0·82
17	0·10	0·0398	0·0400	+0·0002	0·05	3	—
18	0·10	0·0199	0·0199	nil	0·05	3	—
19	0·05	0·1988	0·1990	+0·0002	0·18	1·5	0·75
20	0·50	0·00010	0·00010	nil	—	15*	—
21	0·50	0·00099	0·00100	+0·00001	—	15*	—
22	0·50	0·00249	0·00245	−0·00004	—	15*	0·82
23	0·50	0·00020	0·00020	nil	—	15*	—
24	0·50	0·00050	0·00048	−0·00002	—	15*	—

* Added as sodium tartrate.

The colorimetric method was employed in Experiments 20 to 24 and iodine titration in the others.

It is evident that under the conditions specified a satisfactory separation of arsenic and tungsten is obtained.

In the course of the experiments with small amounts of arsenic and large amounts of tungstic acid it was observed that in the presence of the necessarily high concentrations of tartaric acid, unless added as sodium tartrate (or tartaric acid and sodium hydroxide), the arsenic trisulphide failed to flocculate. Addition of sodium sulphate and sulphuric acid to give the same sodium concentration and pH as with sodium tartrate and sulphuric acid failed to promote flocculation. Experiments carried out with sodium arsenite solutions gave precisely the same results, proving that the phenomenon was due neither to the presence of tungstic acid nor to the necessity for the reduction of quinquevalent to tervalent arsenic prior to its precipitation. The phenomenon is so far unexplained.

Post-precipitation—There is considerable evidence to show that sulphide separations are frequently vitiated by post-precipitation. A number of tests were carried out to determine whether the proposed separation method was so affected. Solutions containing 0·1 g. of tungstic oxide as sodium tungstate and 25 ml. of 0·1 *N* sodium arsenite solution (used to avoid the complicating factor of reduction of quinquevalent to tervalent arsenic) were treated according to the above directions and the arsenic sulphide precipitates collected after various time intervals and washed thoroughly. The precipitates were ignited and the residues examined for tungsten by the tannin phenazone method B (*b*). There was no evidence of post-precipitation (see Table IV).

TABLE IV

Expt.	Time after precipitation, hours	Tungstic oxide in sulphide precipitate, mg.
25	¾	0·02
26	3½	0·05
27	19	0·09
28	26	0·04

(*c*) SEPARATION OF ARSENIC BY DISTILLATION AS TRICHLORIDE—For comparative purposes a few analyses were carried out by the distillation method. Mixtures of tungstic and arsenic acids of known composition were treated with 10 ml. of phosphoric acid (sp.gr. 1·75), 1 g. of sodium bromide and 1 g. of hydrazine sulphate (in place of cuprous chloride advocated by Dieckmann and Hilpert[3]) and distilled with hydrochloric acid, the determination being carried out according to the directions of the A.O.A.C.[10] The results are recorded in Table V.

TABLE V

Expt.	Tungstic oxide taken, g.	Arsenic oxide taken, g.	Arsenic oxide found, g.	Error, g.
29	0·10	0·0992	0·0994	+0·0002
30	0·10	0·0397	0·0395	−0·0002
31	1·0	0·0198	0·0198	nil
32	1·0	0·0080	0·0081	+0·00001
33	2·0	0·00248	0·00247	−0·00001

(*d*) HYPOPHOSPHITE METHOD—B. S. Evans has introduced hypophosphorous acid as a reagent for the separation of a number of elements, including arsenic, successful results being reported in the determination of that element in steel containing tungsten.[11]

TABLE VI

Expt.	Tungstic oxide taken, g.	Arsenic oxide taken, g.	Tungstic oxide in arsenic precipitate
34	1·0	0·0020	0·0606
35	1·0	0·0199	0·0698
36	1·0	0·0040	0·0115
37	0·5	0·0020	0·0064
38	0·25	0·0020	0·0040
39	0·10	0·0020	0·0011
40	1·0	0·0020	0·0025

A number of experiments were carried out to test the completeness of the separation of arsenic and tungstic acids by means of this reagent. Mixtures of known amounts of arsenic acid and tungsten as sodium tungstate were treated by Evans's method, and the finely divided precipitates of arsenic were collected with the aid of filter paper pulp and washed with diluted hydrochloric acid (1 + 3) containing sodium hypophosphite and finally with ammonium chloride solution. The precipitates were then ignited to expel the arsenic. Each yielded a residue of tungstic oxide which was weighed. In Experiments 34 and 35 the tungstic oxide, which was obviously impure (contaminated with arsenic), was dissolved in sodium hydroxide solution and reprecipitated with tannin and phenazone (see B (*b*)). The results are given in Table VI.

As it was not found possible to obtain a quantitative separation of arsenic from tungsten comparable with that by the sulphide and distillation methods, no further work was carried out.

B. SEPARATION AND DETERMINATION OF TUNGSTIC ACID

Two methods only need be considered: (i) the classical acid precipitation; (ii) tannin precipitation. The first, as stated in the preamble to this paper, would be expected to yield low results unless the arsenotungstic acid formed were precipitated by addition of cinchonine. Cinchonine arsenotungstate, unlike the corresponding phosphorus compound, would, however, be expected to lose all or most of the arsenic during ignition (in the presence of carbon from the filter paper). Precipitation by evaporation with acid and addition of cinchonine should in consequence yield satisfactory results in absence of significant amounts of alkali salts.

(*a*) *Acid precipitation*—A solution of sodium tungstate was standardised by precipitating 20-ml. aliquot portions (*a*) by evaporating with perchloric acid,[12] (*b*) by tannin and phenazone, giving the following results: 0·1003, 0·1002, 0·1005 and 0·1004 g.; adopted, 0·1004 g. of WO_3 per 20 ml. A solution of sodium arsenate was also prepared and found by reduction and iodine titration to contain 0·0994 g. of As_2O_5 per 25 ml.

Two series of experiments were carried out, in each of which mixtures of the sodium tungstate solution (equivalent to 0·1004 g. WO_3) and various amounts of the sodium arsenate solution were evaporated to about 20 ml., treated with 50 ml. of concentrated hydrochloric acid and evaporated further to about 10 ml. After addition of 10 ml. of concentrated nitric acid each was evaporated to 5 ml. and then diluted with 50 ml. of hot water; to those of the second series (Expts. 51 to 56) were subsequently added 5 ml. of 5 per cent. cinchonine solution. After standing overnight each precipitate was collected with addition of filter paper pulp, and washed thoroughly; those of the first series (Expts. 41 to 50) were washed with diluted hydrochloric acid (1 in 20), and those of the second series (Expts. 51 to 56) with a solution containing 15 ml. of hydrochloric acid and 60 ml. of 5 per cent. cinchonine solution per litre. The precipitates were then ignited and weighed.

After weighing, the precipitates were dissolved in sodium hydroxide solution and analysed for arsenic, using the sulphide separation (see A (*b*) above) and colorimetric determination by the molybdenum blue method.

TABLE VII

In each experiment, 0·1004 g. of WO_3 was taken

Expt.	Arsenic oxide taken, g.	Tungstic oxide found, g.	Error, g.	Arsenic oxide in ignited precipitate, mg.
		First Series		
41	0·0994	0·0231	—0·0773	0·02
42	0·0994	0·0911	—0·0093	0·14
43	0·0994	0·0554	—0·0450	0·03
44	0·0398	0·0339	—0·0665	0·02
45	0·0199	0·0863	—0·0141	0·15
46	0·0199	0·0750	—0·0254	0·03
47	0·0080	0·0927	—0·0077	0·12
48	0·0080	0·0911	—0·0093	not detected
49	0·0020	0·0995	—0·0009	0·02
50	0·0010	0·0998	—0·0006	0·02
		Second Series		
51	0·0994	0·1000	—0·0004	0·26
52	0·0398	0·0994	—0·0010	0·18
53	0·0398	0·0995	—0·0009	0·10
54	0·0199	0·1000	—0·0004	not detected
55	0·0080	0·0999	—0·0005	not detected
56	0·0020	0·1001	—0·0003	0·06
57	0·0010	0·0998	—0·0006	not detected

The results, given in Table VII, show that, as was to be expected by analogy with phosphoric acid, the presence of arsenic acid prevents the complete precipitation of tungstic acid by mineral acids. Addition of cinchonine improves the recovery. In most experiments the amount of arsenic retained by the tungstic oxide after ignition was insignificant, owing to the reduction of the arsenic compound by the heated carbon and subsequent volatilisation.

To confirm that the cinchonine acts by precipitating cinchonine arsenotungstate, the clear filtrate, after removal of the tungstic acid precipitate in Experiment 45, was treated with cinchonine solution and the precipitate collected and washed. The precipitate was dissolved in ammonia, the solution filtered to remove cinchonine and the arsenic precipitated from tartrate solution with hydrogen sulphide and determined colorimetrically as above (A (*b*)): found, 0·00054 g. of As_2O_5. The tartaric acid was destroyed in the filtrate by wet combustion with sulphuric and nitric acids and the tungstic acid recovered by the tannin phenazone method: found, 0·0127 g. of WO_3, equivalent to 0·00052 g. of As_2O_5 in $24WO_3.As_2O_5$. These results are in good agreement with the hypothesis that the soluble compound produced when a solution of arsenate and tungstate is treated with mineral acid is 12-arsenotungstic acid which is thrown down as its insoluble cinchonine salt on addition of the alkaloid.

(*b*) *Tannin precipitation*—Mixtures of known weights of tungstic oxide and arsenic pentoxide, obtained in solution by fusion with sodium carbonate and dissolution of the melt in hot water, were analysed for tungsten by the tannin cinchonine method.[2] The precipitate, after being ignited and weighed, was analysed for arsenic as above, B (*a*). The results are summarised in Table VIII and show that the tannin phenazone method provides a satisfactory separation of tungstic and arsenic acids.

TABLE VIII

Expt.	Tungstic oxide taken, g.	Arsenic oxide taken, g.	Tungstic oxide found, g.	Error, g.	Arsenic oxide in tungstic oxide, mg.
58	0·1027	0·11	0·1031	+0·0004	0·05
59	0·0519	0·14	0·0522	+0·0003	0·05
60	0·0205	0·18	0·0205	nil	0·10
61	0·0111	0·20	0·0116	+0·0005	0·10
62	0·1518	0·05	0·1519	+0·0001	0·10
63	0·0090	0·50	0·0089	−0·0001	0·05
64	0·0040	0·50	0·0038	−0·0002	0·05
65	0·0998	0·10	0·1008	+0·0010	0·07
66	0·0998	0·10	0·0997	−0·0001	0·10

SUMMARY

A critical study of the methods available for the separation and determination of tungstic and arsenic acids has been made. The distillation method of Dieckmann and Hilpert is considered to be the most satisfactory for the separation of arsenic, although precipitation as sulphide from tartrate solution is capable of yielding equally accurate results. The tannin phenazone method has been shown to give satisfactory results for tungsten in presence of arsenic, whilst the classical acid precipitation method gives low results even when cinchonine is added to the solution.

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January, 1949

Notes

CONDITIONS UNDER WHICH MITES RAISE MOISTURE CONTENT OF FOODSTUFFS

In a recent note, Amos[1] states that mites in flour raised its moisture content from 15·14 to 28·10 per cent. within a period of 18 months, in a jar which was hermetically sealed.

It is readily demonstrated, however, that mites in any appreciable concentration cannot long remain active in a gas-tight container. If samples of mite-infested flour; etc., are enclosed in glass tubes sealed by drawing off the open ends over a flame, the mites become inactive within a few days. Hughes[2] has shown that concentrations of carbon dioxide above 30 per cent. of the atmosphere induce anaesthesia in *Tyroglyphus farinae*, and that 3 days' exposure to pure carbon dioxide kills it. On the assumption that the respiration of the mites can be represented as the complete combustion of polysaccharides to carbon dioxide and water, one can make an estimate of the maximum amount of water the mites could produce under the conditions described by Amos, if the jar were hermetically sealed; the carbon dioxide would be sufficiently concentrated to stop further activity before the moisture content of the flour had been increased by 0·1 per cent. of the flour weight. Although the basis of the calculation is over-simplified, it serves to show the order of magnitude of the maximum change which mites could be expected to bring about under gas-tight sealing.

It seems therefore that the wax seal on the jar used by Amos must have been imperfect.

I have discussed this matter with Dr. Amos, and also with Mr. T. A. Oxley, of this Laboratory, and we are agreed that the correct interpretation of his observations is probably as follows. Mites were able to remain active in a container which was tightly though not hermetically closed, because diurnal and other changes in temperature and atmospheric pressure caused the escape of excess carbon dioxide and the replenishment of the supply of oxygen, even though the fissures in the seal may have been minute. The moisture presumably did not escape to the same extent as the carbon dioxide because much more of it was held by physical sorption by the flour. Had the flour become saturated the accumulation of water could have continued by condensation, which takes place at a relatively low vapour concentration.

Thus, with the proviso that it would not apply to instances of real hermetical sealing, I agree with the submission made by Amos[1], "that the excess moisture was the natural outcome of the metabolic activity of the mites proceeding in an enclosed space." He has demonstrated the important fact that, if mites are present, good but imperfect sealing up of materials may shut in much more moisture than it keeps out.

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March, 1949

THE RECOVERY OF URANIUM FROM SODIUM ESTIMATION RESIDUES

The Board of Trade have recently stated[1] that no further quantities of uranium compounds can be supplied to dealers for many months to come; workers in laboratories using uranium compounds must therefore conserve any quantities they may already have.

In laboratory routine, uranyl acetate is often used for determining sodium as sodium zinc (or magnesium) uranyl acetate. During the war a method of recovering uranium from residues and liquors left after sodium determinations with zinc uranyl acetate was developed in the Chemical Inspection Department of the Ministry of Supply. This method may be of interest to other users of uranium compounds at the present time. Although it was used only for residues from estimations in which zinc uranyl acetate was employed, it could presumably be applied to those obtained with magnesium uranyl acetate.

The principle of the method is the precipitation[2] of hydrated uranium peroxide, $UO_4.2H_2O$, which is converted into uranium trioxide, UO_3, by ignition.[3,4,5] The trioxide dissolves readily in acetic acid, forming uranyl acetate, the salt required for preparing the precipitating agent for sodium. Liquid and solid residues are collected and treated separately; the liquid residues are collected in Winchester bottles and, in order to prevent photo-reduction to green uranous salts, are stored in the dark until required for treatment.

Method—For the treatment of liquid residues, separate the lower, aqueous layer from the upper layer comprising organic solvents, and filter it through a Buchner funnel. Transfer 500 ml. of the filtrate to a 2-litre beaker and heat on a water-bath until most of the ether is removed. Add about three times the volume of hot water and transfer the beaker to a hot plate.

For solid residues, dissolve 20 g. of the solid in about 1½ litres of hot water in a beaker.

To the hot solution thus obtained, whether from liquid or from solid residues, run in slowly from a pipette about 100 ml. of 10-vol. hydrogen peroxide and stir constantly. A heavy yellowish-white precipitate of $UO_4.2H_2O$ is formed. Boil the liquid for 10 minutes and then transfer the beaker to the water-bath and allow the precipitate to settle. If the supernatant liquid is colourless, precipitation of the uranium is complete, but if it is yellow, add more hydrogen peroxide and repeat the boiling and settling.

Remove the clear supernatant liquid from the beaker by suction. Wash the heavy precipitate in the following manner. Add about 1 litre of hot water, boil for a few minutes, and allow to settle. Almost the whole of the precipitate settles in about 2 or 3 minutes, but without waiting for complete settling suck off the supernatant liquor. Continue washing the main bulk of the precipitate until the zinc has been removed, as shown by testing the washings with ammonium sulphide. About 4 or 5 washings are required. The slightly cloudy supernatant liquid washings may be collected and the precipitate suspended therein allowed to settle.

Dry the washed hydrated uranium peroxide precipitate as far as possible by placing the beaker containing it on a water-bath or low-heat hot plate. Transfer the nearly dry solid to a silica dish and, after crushing the lumps, heat it on a high-heat hot plate, with occasional stirring, until it is of a uniform orange colour and a test portion is completely soluble in dilute acetic acid. The orange solid is uranium trioxide, UO_3; the heating takes $\frac{1}{2}$ to 1 hour, and if care is exercised it may be carried out entirely in the original beaker. If the mass begins to turn green the temperature is too high; reduce the heat and see that there is free access of air. The green colour is that of urano-uranic oxide, U_3O_8, which is insoluble in acetic acid and therefore of little use in the recovery process. However, it is not likely that U_3O_8 will be formed, even at the temperature of a high-heat hot plate.

Uranium trioxide recovered by the above method will, if the washing has been carried out efficiently, be completely free from zinc. Very small quantities of uranium peroxide and of the oxide U_3O_8 may be present, but these remain insoluble when the trioxide is dissolved in dilute acetic acid and are removed by filtration.

The author wishes to record his thanks to Messrs. R. F. Callen and T. Moodie for their assistance in working out the method; also he thanks the Chief Scientist, Ministry of Supply, for permission to publish this note.

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April, 1949

THE TUNGSTIC ACID PRECIPITATION OF PROTEIN FROM PHYSIOLOGICAL FLUIDS

(1) It has been found that in order to precipitate proteins from the blood of patients who have been treated with alkaline mixtures for a month or more it is necessary to use more than 1 ml. of 2/3 *N* sulphuric acid per 1 ml. of 10 per cent. sodium tungstate solution with 1 ml. of blood and 7 ml. of water. As a rule, 0·2 ml. of extra sulphuric acid, with 6·8 ml. of water, are sufficient.

(2) One ml. of tears with 8·4 ml. of water, 0·25 ml. of 10 per cent. sodium tungstate solution and 0·35 ml. of 2/3 *N* sulphuric acid, shaken and filtered through a Whatman No. 1 paper, give a clear solution in which urea plus ammonia can be estimated by the Lieboff method.

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Erratum: June (1949) issue, p. 362.

Equation (iv), line 14 from the bottom of the page, should read

$$W = \frac{ap}{T_9} - bD \quad \ldots \quad \text{(iv)}$$

Order in Council

STATUTORY INSTRUMENT*

1949—No. 1047 (S. 68). **The Transfer of Functions (Food and Drugs) (Scotland) Order, 1949** Price 1d.

This Order, which applies only to Scotland and came into force on June 20th, 1949, *transfers to the Secretary of State for Scotland acting jointly with the Minister of Food the powers of the Secretary of State under the Food and Drugs* (*Adulteration*) *Act,* 1928, *to make regulations as regards the qualifications of public analysts, and under the Food and Drugs Act,* 1938, *to make regulations concerning food, other than meat from home-fed animals and milk, its composition and the conditions under which it is imported, prepared, transported, stored or sold.*

The Order also transfers to the Minister of Food (a) *the power of the Secretary of State to make regulations under the Food and Drugs* (*Adulteration*) *Act,* 1928, *as regards the presumptive evidence of adulteration of condensed milk, cream, butter or cheese*; (b) *the power of the Secretary of State to make regulations as regards dried or condensed milks, including in particular their composition and the labelling of receptacles containing them*; *and* (c) *certain administrative functions exercised at present by the Secretary of State under the Food and Drugs* (*Adulteration*) *Act,* 1928, *and the Food and Drugs Act,* 1938, *relating to food, including in particular functions relating to margarine, margarine-cheese and milk-blended butter and the importation of food.*

Ministry of Food

STATUTORY INSTRUMENTS*

1949—No. 1378. The Soft Drinks (Amendment) Order, 1949. Price 1d.

This Order, as from July 31*st,* 1949, *prescribes specifications for ingredients of "lime juice and soda."*

—No. 1497. The Shredded Suet Order, 1949. Price 1d.

In this Order, "shredded suet" includes flaked suet but does not include any product which is of vegetable origin or any product having fibrous tissues.

The Order provides (a) *that all manufacturers and pre-packers of shredded suet must be licensed*; *and* (b) *that shredded suet must not be sold at a price exceeding the price stated upon a label attached to the wrapper or container.*

—No. 1498. The Manufactured and Pre-packed Foods (Revocation) Order, 1949. Price 1d.

As from August 7th, 1949, *this Order revokes the Manufactured and Pre-packed Foods* (*Control*) *Order,* 1942, *as amended* (*S.R. & O.,* 1942, *Nos.* 1863 *and* 2073; 1944, *No.* 167; 1945, *No.* 250; 1946, *No.* 1486; and S.I., 1948, No. 131), *but without prejudice to any proceedings in respect thereof.*

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Grading of Raw Milk by Means of the Resazurin Test. T. E. Galesloot (*Ned. Melk Zuiveltijdschr.*, 1948, **2**, 70–90)—Because of the increasing interest in the use of the resazurin test as a means of grading raw milk, a comparison of this test and the methylene blue test was undertaken. The author concludes that the resazurin test is not a satisfactory method of determining the quality of raw milk.

The resazurin test was carried out by adding to 10 ml. of milk 1 ml. of a 0·005 per cent. resazurin solution, and standing the tubes in a water-bath at 37° C. The colour of the sample was estimated after 1 hr. for summer milk and after 2 hr. for winter milk; the English colour scale was used, in which 6 indicates a sample in which no colour change has occurred, 5 to 2 indicate shades between blue and pink, 1 is pink, and 0 is white. The methylene blue test was conducted in the usual way, the tubes being inverted after each hour. The catalase number was also determined for the winter samples, as a measure of cell activity. The cell content was estimated by means of a plate count, using meat extract - tryptone - glucose - skim milk - agar, and incubating the plates for 3 days at 30° C.

540 samples of winter milk were examined, 180 of morning milk, 180 of 12-hr. old evening milk, and 180 of mixed milk. Very poor agreement was found between the resazurin numbers and the methylene blue reduction times. The resazurin test appears to be very considerably affected by the tissue cells in the milk, and the author does not agree that milks with a high cell activity are always pathologically or physiologically abnormal, as mastitis seldom occurred in the herds from which the milk samples were taken.

Storage at 20° C. for 5 hr., followed by 16 hr. in the ice-box, shortened the methylene blue time, whilst the resazurin test showed variable results, some samples having the same resazurin number, others greater and others smaller, than before storage. With samples that had the same resazurin number before and after storage, there was a relationship between the catalase values and the resazurin numbers before storage, and there was also a correlation between the catalase numbers and the alteration in resazurin number on storage.

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* Obtainable from H.M. Stationery Office.

Agreement between plate counts and resazurin results was much worse than between plate counts and methylene blue reduction times, although after storage there was a better agreement between plate count and resazurin value.

184 samples of summer morning milk were investigated, though not in as great detail as the winter milk. The agreement between methylene blue reduction times and resazurin values was better than with winter milk, this being explained by the fact that in summer milk there are fewer tissue cells and more bacteria, but again, the results obtained with the resazurin test are unduly affected by the cell activity of the milk. Storage for 4 hr. at room temperature (above 20° C.) reduced the methylene blue times and generally reduced the resazurin values, but the results for the latter test were variable. The agreement between plate count and methylene blue reduction time was better with summer milk than that between plate count and resazurin test results, samples with low plate counts often showing low resazurin numbers, so that milk of good quality could not be detected by this latter test.

The effect of temperature on the two tests was seen by comparing the results for winter and summer milk. With both warm and cold milk there is no great difference in the relation between plate count and methylene blue reduction time. With the resazurin test, the temperature of the milk has a much greater influence on the values obtained, and the relationship between plate count and resazurin number is also affected by temperature.

The conclusion to be drawn is that the tissue cells affect the reduction of resazurin to such an extent that there is no satisfactory agreement between the resazurin values and the quality of the milk.

E. M. POPE

Estimation of Milk Proteins in Milk. E. Gorffon (*Le Lait*, 1948, **28**, 449–454)—The method is based on the colour developed with Folin and Ciocalteu's reagent (*J. Biol. Chem.*, 1927, **73**, 629) by the tyrosine and tryptophan contained in milk proteins. To calibrate the colorimeter or photo-colorimeter employed, commercial casein is not used as it is not wholly soluble under the conditions of the test, especially if old. A phenolic body that can be obtained pure and dry and is readily soluble is preferred, and by the method described a 0·048 per cent. resorcinol solution gives the same colour as a 1 per cent. solution of milk proteins. The method is as accurate as available macro-methods. Since lactalbumin as well as casein is determined, there will be an error of about 1 in 15; this is negligible in an industrial method such as is proposed. The determination can be made in 20 to 25 min.

Procedure—Shake 1 ml. of milk with 4 ml. of alcoholic sodium hydroxide (0·5 g. in 100 ml. of alcohol at 60° C.) and then with 5 ml. of ether until the characteristic opalescence of the milk has disappeared. Add water dropwise and with constant shaking until the aqueous phase measures 10 ml.; withdraw 1·5 ml. of this and dilute it with 25 ml. of water. Add 1 ml. of alcoholic sodium hydroxide followed rapidly by 3 ml. of Folin and Ciocalteu's reagent and water to 50 ml. After 5 to 10 min., measure the blue colour on a colorimeter or photo-colorimeter, using a red filter.

A. H. ADAMS

Component Acids and Glycerides of Whale Oil. T. P. Hilditch and L. Maddison (*J. Soc. Chem. Ind.*, 1948, **67**, 253–257)—The component acids of a specimen of Antarctic whale oil, previously (*Ibid.*, 1942, **61**, 169) separated by means of their lithium and lead salts before ester-fractionation, have now been separated by low-temperature crystallisation from solvents. The results agree well with those obtained by the other method. Six groups of component glycerides were separated by crystallisation from acetone from −60° C. upwards. The results were generally similar to, but with some numerical difference from, those by the lithium - lead salt method (*loc. cit.*); the oil contains approximately 16 per cent. of di-saturated and 2·5 per cent. of trisaturated glycerides, about 30 per cent. of tri-unsaturated glycerides and about 50 per cent. of glycerides containing one saturated acid, one unsaturated C_{18} acid, and one of the other homologous unsaturated acids. Acids of the C_{20} and C_{22} series are contained in about 45 per cent., and oleic groups in over 90 per cent., of the oil. Iodine values of the different oil fractions are: insoluble at −10° C., 29·1; soluble at −10° C., 63·2; soluble at −20° C., 82·7; soluble at −40° C., 118·3; soluble at −60° C., 215·2; soluble at −60° C. (recrystallisation), 192·9.

Component acids are calculated as:—

	By lithium and lead salt method per cent. (wt.)	By low-temperature crystallisation (present work)	
		(*a*)	(*b*)
Lauric	0·2	Trace	0·3
Myristic	9·3	9·2	9·3
Palmitic	15·6	15·6	15·6
Stearic	2·8	1·9	2·3
Arachidic	0·3	0·6	0·2
Unsaturated C_{14}	2·5 (− 2·0)	2·5 (−2·6)	2·6 (−2·0)
,, C_{16}	14·4 (− 2·1)	13·9 (−2·1)	13·8 (−2·1)
,, C_{18}	35·2 (− 2·5)	37·2 (−2·4)	36·9 (−2·4)
,, C_{20}	13·6 (− 7·2)	12·0 (−7·1)	12·2 (−7·0)
,, C_{22}	5·9 (−10·1)	7·1 (−9·4)	6·8 (−9·8)
,, C_{24}	0·2 (−10·4)	—	—

E. B. DAW

Investigations of Some New Sudan Seed Oils. D. N. Grindley (*J. Soc. Chem. Ind.*, 1948, 67, 230–231)—The seed oils of members of the families Ulmaceae {(i) *Celtis integrifola*, (ii) *Chaetacme microcarpa*, (iii) *Trema guuneensis*}; Capparidaceae {(iv) *Boscia octandra*}; Rhamnaceae {(v) *Zizyphus Spina-Christi*}; Tiliaceae {(vi) *Grewia villosa*}; Euphorbiaceae {(vii) *Chrozophora plicata*}; and Salvadoraceae {(viii) *Dobera roxburghii*} were examined.

Results:—

Species	(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)
Wt. of 100 seeds, g.	31·8	22·1	0·65	20·89	4·29	5·34	1·98
Oil in material, %	6·12	6·39	28·08	0·91	29·37	0·81	38·47
Oil constants:							
Sapon. value	187·1	189·7	191·4	175·2	188·0	184·6	195·2
Unsaponifiable, %	2·09	2·58	2·68	9·96	1·11	3·90	0·70
Insol. F.A. + unsap. (Hehner)	—	96·59	95·08	—	94·80	—	95·0
Fatty acids constants:							
Iodine value (g. I/100 g.)	144·0	152·6	116·8	76·5	89·28	113·4	112·5
Thiocyanogen value (g. I/100 g.)	82·1	83·8	72·3	54·0	69·1	78·2	71·4
Mean mol. wt.	280·9	280·7	276·3	275·7	281·3	279·5	272·8
Saturated acids, %	13·6	12·4	23·3	41·8	24·9	15·9	24·0
Mean mol. wt. of sat. acids	285·0	284·8	262·9	268·4	281·0	271·2	250·0
Composition of fatty acids:							
Linoleic acid, %	73·7	82·0	53·0	26·8	24·0	41·8	48·9
Oleic acid, %	12·7	5·6	23·7	31·4	51·1	42·3	27·1
Stearic acid, %	13·6	12·4	6·2	41·8	22·6	15·9	2·04
Palmitic acid, %	—	—	17·1		2·3		

Petroleum spirit extraction of kernels of (viii) yielded 0·63 per cent. of oil; further extraction with chloroform gave 1·0 per cent. more—of very bad odour. The first extract had a saponification value 130·6, and contained 30·0 per cent. of unsaponifiable matter. The mean mol. wt. of combined fatty acids was 288; the iodine value of the petroleum spirit extract was 54·5. This oil differs greatly from those of seeds of *Salvadora persica* and *S. oleoides* (of the same family), which contain high percentages of oils characterised by large amounts of acids of low molecular weight, especially myristic.

E. B. Daw

The Roeder Method for Determining Fat in Cheese. M. J. Bernaerts (*Ned. Melk Zuiveltijdschr.*, 1948, 2, 99–107)—Following the appearance of a new type of cheese butyrometer, marketed by the Gerber firm, and described as the *Käsebutyrometer nach Dr. Roeder*, an investigation was made of the Roeder method of fat determination in cheese (Gerber, *Traité pratique des Essais du Lait et du Contrôle des Produits Laitiers*, 9th Edn., revised by Charles Schneider, Berne, 1941, p. 105). This method is as follows. Weigh out 2·266 g. of cheese into a small beaker with perforated walls, place the beaker in the butyrometer, and cover the cheese with 15 ml. of solvent; this solvent is prepared by dissolving 43 g. of stannous chloride in 700 ml. of concentrated hydrochloric acid and diluting to 1 litre with water. Heat for 10 to 15 min. in boiling water, shaking the butyrometer several times. Add 1 ml. of amyl alcohol and sufficient of the reagent to bring the meniscus to the upper part of the graduated portion of the neck. Shake vigorously once more and centrifuge at 1000 revolutions per min. for 5 min. Read the percentage at 65° to 70° C. The results obtained by this method were always too high, compared with those obtained by the Van Gulik and Weibull methods; particularly high figures were obtained with highly fermented cheeses. The cause of the deviations was found to be the formation of amyl ether and of amyl esters of lower fatty acids; these reactions proceed much more rapidly in presence of the stannous chloride solution than in presence of the dilute sulphuric acid used in the other two methods. Better results were obtained by (i) cooling the butyrometer to 65° C., after the cheese had dissolved and before adding amyl alcohol, as the formation of amyl ether and esters is quicker at the boiling-point, and (ii) by weighing out a smaller quantity of cheese, 2·09 g. The centrifuging must be done immediately after the amyl alcohol is added and the butyrometer shaken.

E. M. Pope

Component Acids of Rape Seed Oil. M. N. Baliga and T. P. Hilditch (*J. Soc. Chem. Ind.*, 1948, 67, 258–262)—For preliminary separation of rape and similar cruciferous seed oil fatty acids before ester-fractionation, crystallisation from 10 per cent. solution in ether at −40° C. for 5 hr. was followed by recrystallisation (as before) of the deposited acids. Fractions are distinguished as (*A*) insoluble at −40° C., (*B*) soluble at −40° C., and (B^1) soluble at −40° C. (from recrystallisation of (*A*)). This method permits the approximate determination of the three unsaturated minor component acids—hexadecenoic, eicosenoic, and docosadienoic—and five saturated ones—palmitic, stearic, arachidic, behenic, and lignoceric—besides the four major component acids—erucic, oleic, linoleic, and linolenic. The average percentage fatty acid composition of four rape seed oils—Indian (Toria, Guzerat), Polish (Danzig), and Argentine (Plate) is palmitic 2·5, saturated C_{18}, C_{20}, C_{22}, and C_{24} (together) 5, hexadecenoic 2, oleic 15, linoleic 13·5, linolenic 8, eicosenoic 5, erucic 48, and docosadienoic 1 (wt.). Ravison (Black Sea) seed oil (*A*) and Jamba (Indian) rape seed oil (*B*) had the following percentage (wt.) composition: palmitic (*A*) 4, (*B*) 4·5; saturated

C_{18} to C_{24} (*A*) 5, (*B*) 5; hexadecenoic (*A*) 0·5, (*B*) 1·5; oleic (*A*) 15·5, (*B*) 24; linoleic (*A*) 21, (*B*) 9; linolenic (*A*) 10, (*B*) 6·5; eicosenoic (*A*) 4, (*B*) 11·5; erucic (*A*) 39, (*B*) 37·5; docosadienoic (*A*) 1, (*B*) 0·5.
E. B. DAW

Biochemical

Separation of Acidic Amino Acids by Means of Synthetic Anion Exchange Resin. R. Consden, A. H. Gordon, and A. J. P. Martin (*Biochem. J.*, 1948, **42**, 443–447)—A chromatographic method is presented for separating glutamic from aspartic acid after their separation together from a protein hydrolysate, the synthetic anion-exchange resin Amberlite IR4 being used.

*Method—Preparation of Amberlite IR*4 (Resinous Products and Chemical Co., Pa.)—Alternately grind the resin wet in a ball mill and sieve with a jet of water. Use −90 + 200 grading for the preparation of absorption columns. Wash free from ammonia, treat with hydrochloric acid until the supernatant liquid is permanently acid to thymol blue (pH <1), and wash with water. Treat alternately a number of times with acid and water.

Preparation of columns—(1) Stir and wash the resin by decantation with water until the supernatant liquor is less than 0·001 *N* with respect to alkali. Transfer the slurry to a glass tube of 0·5 cm. internal diameter, forming a column 20 cm. in height and containing about 2 g. of air-dry resin. Wash overnight with water at about 15 ml. per hr. This does not significantly raise the pH of the eluate, the acid concentration of which should be checked by titration before the next stage. (2) Prepare the second column (height 30 cm.) from about 3 g. of Amberlite and adjust the pH accurately to 2·5 by equilibration with dilute acid and water until the acidity of the supernatant fluid is slightly above 0·003 *N*. Transfer the material to a 0·5-cm. diameter tube and wash overnight with 0·003 *N* hydrochloric acid. Finally, check the acidity of the eluate by titration. The columns should not be allowed to run dry and remain so for any length of time or extensive cracking may occur.

Procedure for the estimation of glutamic and aspartic acids—Prepare the hydrolysate, containing up to 15 mg. of acidic amino acids, with hydrochloric acid, repeatedly evaporate *in vacuo* by rotating manually in a warm water-bath, allow to stand overnight *in vacuo* over potassium hydroxide, and quantitatively transfer in a small volume to the first column. Pass water down the column and elute the neutral and basic amino acids in the first 25 ml. The next 5 ml. should be free from amino acids. To test the eluate concentrate a portion of the first 25 ml. and all of the second 5 ml. fraction separately *in vacuo* to a very small bulk and test separate portions of each by partition chromatography on a strip of Whatman No. 4 paper, using phenol-ammonia, developing for 5 hr. or more, with a suitable control (*cf. Ibid.*, 1944, **38**, 224). After development and treatment with ninhydrin, basic and neutral but no acidic amino acids are found in the first eluate, whilst the second eluate should contain no amino acids. If cystine is present, oxidise the eluate sample with an excess of bromine water before chromatography; the cysteic acid thus produced occupies a position on the chromatogram not overlapping the positions of aspartic and glutamic acids (*Ibid.*, 1946, **40**, 580) if these are present. Next transfer the material remaining on the column to the second column by either of the following methods.

Method 1—Elute the column with *N* hydrochloric acid, causing the pigment to run down in a sharp band. When this has run out, stop washing, and repeatedly evaporate the eluate *in vacuo* and allow to stand overnight *in vacuo* over potassium hydroxide to remove the excess of hydrochloric acid. Transfer quantitatively to the second column, which is eluted with 0·003 *N* hydrochloric acid. Collect 10-ml. eluates and concentrate each *in vacuo* to 1 to 2 ml. and test a 4-μl. sample as described above.

Method 2—Wash the column with 3 ml. of *N* hydrochloric acid, causing the pigment to travel about halfway down, and testing that the eluate is free from amino acids by chromatography. Fix the column to the top of the second column, and continue the elution with water. The acid already added is sufficient to cause the pigment band to continue to travel down and eventually to pass into the lower column. When the pigment has travelled a few centimetres into the second column, remove the top column and continue the elution with 0·003 *N* hydrochloric acid. If no pigment is present elution may be followed by the change in shade of the resin, which is orange when strongly acid. Collect the eluates and test as in Method 1.

Method 1 gives more consistent results than Method 2. In proteins where the ratio of glutamic acid to aspartic acid is of the order of 2 to 1, the glutamic acid is eluted from the second column in the first 20 ml., and aspartic acid in the next 30 ml. In Method 2 the stages at which the acids begin to appear is less certain. Method 2 is quicker than Method 1, but requires more attention.

Evaporate each eluate *in vacuo* to dryness and add to the crystalline residue a known volume (5 to 10 ml.) of copper phosphate buffer suspension (*Ibid.*, 1939, **33**, 1070). Titrations (20 to 50 μl. of 1·0 *N* sodium thiosulphate) are carried out on 1-ml. portions after filtering and addition of the other reagents. Neutralisation before the addition of the copper phosphate is unnecessary.

Recoveries of glutamic and aspartic acids from a synthetic mixture by this technique varied from 88·8 to 97·6 per cent.

A method for isolating cysteic acid from wool hydrolysates is also presented. J. S. HARRISON

Chromatographic Separation of Amino Acids and Peptides. I. Chromatography of Neutral Peptides in 10 per cent. Formol. E. Lederer and Tchen Pau Kium (*Biochimica et Biophys. Acta*, 1947, **1**, 35–41)—Neutral di- and tripeptides can be separated quantitatively from other neutral non-adsorbed amino acids in 10 per cent. formol solution by adsorption on acid-activated alumina. The adsorbed amino acids are quantitatively eluted in an alkaline medium.

β-Alanine can also be separated quantitatively from its α-isomer and a method is described in which *o*- and *p*-aminobenzoic acids are adsorbed on activated alumina in absence of formol.

Peptides—The apparent acid dissociation constant (pKa′) of amino acids is displaced by addition of formol. For example, the pKa′ of glycine in water is 9·60, and 5·92 in 9 per cent. formol solution, and that of serine is 9·15 in water, and 5·63 in formol. Dunn and Loshakoff (*J. Biol. Chem.*, 1936, **113**, 691) found that certain peptides resembled glycine and serine in respect of their pKa′ in formol solution, *e.g.*, the pKa′ of glycyl-glycine is changed from 8·13 in water to 4·27 in 9 per cent. formol solution. It was therefore likely that neutral peptides would, like glycine and serine, be adsorbed on acid-activated alumina. This was confirmed and it was found that neutral di- and tripeptides also were adsorbed. This provides a method for the quantitative separation of the amino acids and neutral peptides, although its usefulness is diminished by the fact that glycine, serine, threonine, and cysteine are adsorbed with the peptides. The only other practical method, that of E. Fischer and E. Abderhalden (*Z. Physiol. Chem.*, 1905, **46**, 52), is not suitable for quantitative application.

β-Alanine—As the effect of formol on the acidity of glycine is probably due to the presence of an $-NH_2$ group attached to a primary carbon, similar effects can be expected with all the ω-amino acids. This effect was verified. β-Alanine in 10 per cent. formol solution is quantitatively retained on an acid-activated alumina column and can be separated quantitatively from the α-isomer. The pKa′ of β-alanine is 6·41 in 10 per cent. formol solution, compared with 7·26 for α-alanine. γ- and δ-Amino acids containing the $-CH_2NH_2$ group behave in a similar way to β-alanine.

Aminobenzoic acids—Both *o*- and *p*-aminobenzoic acids can be adsorbed on acid-activated alumina, even in absence of formol.

General procedure—Essentially, the technique of Schramm and Primosigh (*Ber.*, 1943, **76**, 373) is followed. Dissolve 3 to 30 mg. of amino acid or peptide in 5 ml. of 10 per cent. formol solution, purified by distillation of commercial formaldehyde: the distillate, free from nitrogen, contains about 30 per cent. of formol. Make a convenient dilution and neutralise just to the rose colour of phenolphthalein (pH about 8·5). Pass the solution of the substance to be tested through a column of 20 g. of acid-activated Merck alumina, prepared according to Wieland (*Z. physiol. Chem.*, 1942, **273**, 24; *Ber.*, 1942, **75**, 340 and 1001), and previously washed with 75 ml. of 10 per cent. formol solution. After the 5 ml. of solution have penetrated into the column, pour thereon about 50 ml. of 10 per cent. formol solution. The filtrate contains the substances that pass through the column without being adsorbed. Elute the adsorbed substances by washing the column with dilute alkali; use about 20 ml. of 0·5 *N* potassium hydroxide for neutralising the acid in the column, followed by 30 ml. of 0·1 *N* potassium hydroxide to effect the elution in feebly alkaline solution. Collect the alkaline liquid in an excess of dilute sulphuric acid. The elutions can also be carried out with aqueous baryta, as this facilitates subsequent treatment of the eluate after precipitation of the baryta with sulphuric acid (*cf.* Wieland, *loc. cit.*).

The recoveries of glycine, DL-serine, β-alanine and all the di- and tripeptides tested were between 95 and 102 per cent., whereas α-alanine and DL-leucine were not retained on the column. It was considered unnecessary to try other neutral amino acids, which were known to pass through the column without being adsorbed. Where 5 ml. of an aqueous solution containing 4·4 mg. of *p*-aminobenzoic acid at pH 6·5 was passed through 20 g. of acid-activated alumina and washed with 75 ml. of water, followed by 50 ml. more of water, all the acid was retained, but could be eluted quantitatively with dilute alkali. Anthranilic acid behaved similarly.

J. S. HARRISON

Photometric Estimation of Purine and Pyrimidine Nucleosides by Means of the Reaction with Orcinol. L. Massart and J. Hoste (*Biochimica et Biophys. Acta*, 1947, **1**, 83–86) A method is described for estimating pyrimidine nucleosides by means of the reaction of Bial, as modified according to Barrenscheen and Peham (*Z. physiol. Chem.*, 1942, **272**, 81), in presence of purine nucleosides. In the normal condition of the reaction with orcinol the purine nucleosides are not hydrolysed. Treatment with bromine water or catalytic reduction diminishes the resistance to hydrolysis.

Estimation of pure pentoses and pure purine nucleosides—Mix equal volumes (3 ml.) of the solution to be tested and an orcinol reagent prepared at the time of the determination by dissolving 0·2 g. of orcinol in 100 ml. of a solution of $M/2500$ cupric chloride in hydrochloric acid. (This reagent is stable for several hours.) Allow to stand for 10 min. in vigorously boiling water and cool as quickly as possible in running water. Measure the extinction in a 1-cm. cuvette, using a Leitz photometer with filter 620, or a Zeiss instrument with filter 610.

Results—Extinction coefficients determined for xylose, arabinose, adenosine, and adenosine triphosphoric acid agreed well with those of Barrenscheen and Peham, except that the values for xylose were slightly greater than those for arabinose, and the values for adenosine, freed from pentose, were slightly high, but those for adenosine triphosphoric acid agreed well.

Hydrolysis—The method of hydrolysis used by Barrenscheen and Peham was not satisfactory for the hydrolysis of pyrimidine nucleoside unless preceded by treatment with bromine. The bromine must not be allowed to act for too long or the colour produced is unsuitable for colorimetric estimation. The following method was adopted for adenosine and cystidine: add 0·01 ml. of bromine to 3 ml. of a solution containing the nucleosides, place in an oil-bath at 105° C., and stir mechanically with a glass stirrer. After 2 min., remove the excess of bromine by a current of air and then replace in the oil-bath for 38 min. After this time add an equal volume of the orcinol reagent and allow to react for 10 min. in the oil-bath at 105° C. Cool quickly in running water and measure the extinctions. This method gave reproducible results.

Extinction of pyrimidine nucleoside in presence of purine nucleosides—On one portion of the sample measure the purine nucleoside by the method of Barrenscheen and Peham to obtain a value *a*, corresponding to an extinction *b* for the bromine method described, using a graph constructed by joining the points 100 μg. of nucleoside against 0·20 extinction, and 300 μg. against 0·38 extinction, by a straight line. On a second portion of the solution determine the extinction by the bromine method described, thus obtaining a value *c* for pyrimidine nucleosides + purine nucleosides. The content of pyrimidine nucleoside is obtained by subtracting *b* from *c*. This method can be applied to the quantitative measurement of mixtures of pyrimidine nucleosides and purine nucleosides in extracts of animal organs. J. S. Harrison

Determination of Peptide-Amino Nitrogen by the Copper Method. H. K. Kerkkonen (*Acta Chemica Scandinavica*, 1948, **2**, 518–522)—Solutions of partially hydrolysed proteins give too high results for amino-nitrogen by Pope and Stevens's method (*Biochem. J.*, 1939, **33**, 1070), indicating that the soluble compounds formed by copper with peptides are not similar to those formed with amino acids.

Experiments with glycyl-L-leucine, glycyl-glycine, L-leucyl-L-tyrosine and L-leucyl-glycyl-glycine, alone or in admixture, showed that the Van Slyke method gave about 50 per cent. of the total nitrogen (33·3 per cent. with L-leucyl-glycyl-glycine) as would be expected, whereas the Pope and Stevens method gave 97·2 to 98·5 per cent. (66·6 per cent. for L-leucyl-glycyl-glycine). Confirmatory evidence was also obtained in examining a pepsin-digested zein solution, an acid digest of zein plastein (Virtanen and Kerkkonen, *Nature*, 1948, **161**, 888) as well as with experiments on pentaglycine ethyl ester and glycyl-tyrosine methyl ester.

Woiwod (*Biochem. J.*, 1948, **42**, xxviii) has reported results for the α-amino-N/copper ratio of a number of amino acids, using a colorimetric copper determination, the ratio being twice that found for the dipeptide alanyl-glycine.

When titrating the peptide-copper compound, therefore, 1 ml. of 0·01 *N* sodium thiosulphate is equivalent to 0·14 mg. of amino-nitrogen and not to 0·28 mg. as for amino acids. D. C. M. Adamson

Colorimetric Estimation of Hexoses with Carbazole. G. Holzman, R. V. MacAllister, and C. Niemann (*J. Biol. Chem.*, 1947, **171**, 27–35)—Hexoses and pentoses, heated with sulphuric acid, produce a colour in presence of carbazole. The optimum conditions of this reaction for estimating hexoses have been found.

Reagents—Purify the carbazole by precipitating it three times with cold water from a solution in concentrated sulphuric acid. Dry the product and crystallise from toluene. To obtain low blanks, heat sulphuric acid under reflux with potassium persulphate (20 mg. per litre) until a negative test for oxidising agents is obtained with starch-iodide. *Carbazole reagent*—To 300 ml. of 84 weight per cent. sulphuric acid, add 10 ml. of a 1 per cent. solution of carbazole in absolute ethanol. This reagent is stable for only about 6 hr. and should be freshly prepared every day.

Procedure—Place 9-ml. aliquots of the carbazole reagent in tubes kept in an ice-bath. Add 1-ml. aliquots of hexose solution containing from 50 μg. to 150 μg. of glucose. Mix well and then heat in boiling water for 15 min. Cool in an ice-bath and determine the colour in a Klett colorimeter, using the green filter No. 54.

The accuracy of the method is 2 to 5 per cent. and duplicate samples should be taken. The colour intensity varies with the concentration of sulphuric acid used, reaching a maximum at 84 per cent. by weight. Increasing the carbazole concentration increases the colour intensity. The time of heating is not critical if it is between 10 and 20 min. Sodium nitrate, sodium nitrite, or ferric chloride (in amounts of 5 μg.) did not interfere with the determination of 100 μg. of glucose; N-acetyl glucosamine did not produce a colour.

The extinction coefficients of the colours with glucose, fructose, galactose, and mannose were different, but not so much so that a qualitative identification of the sugars would be possible.

The mechanism of the reaction is discussed in terms of the ultra-violet spectra of sulphuric acid solutions of hexoses. W. S. Wise

Colour Reaction for Glucuronic Acid. Z. Dische (*J. Biol. Chem.*, 1947, **171**, 725–730)—A colour reaction with thioglycollic acid is described for the detection of glucuronic acid.

Procedure—To 0·8 ml. of a solution containing 0·02 to 0·04 per cent. of glucuronic acid, add 0·2 ml. of a 0·2 per cent. solution of mannose. Add, with cooling and shaking, 4·5 ml. of a mixture of diluted sulphuric acid (6 + 1). Keep for 2 min. in ice-water, then in water at 20° to 25° C. and, later, keep for 3 min. in boiling water. Cool to room temperature and add 0·1 ml. of a 2·5 per cent. solution of commercial thioglycollic acid. Shake well and keep at room temperature for 20 to 24 hr. A deep pink colour is produced.

A colour is produced by hexuronic acids and other compounds when treated with thioglycollic acid and concentrated sulphuric acid. The colour produced in presence of an excess of mannose appears to be selective for glucuronic acids and polyglucuronides.

The absorption spectra of the colours produced by some hexuronic acids and polyuronides are given. W. S. Wise

Determination of *p*-Aminosalicylic Acid in Blood and Cerebrospinal Fluid. W. Klyne and J. P. Newhouse (*Lancet*, 1948, **255**, 611)—Although Lehman (*Svenska Läkartidn*, 1946, **43**, 2029) mentioned Ehrlich's reagent for the determination of *p*-aminosalicylic acid in blood, no details were given. A method based on Morris's procedure for the determination of sulphanilamide (*Biochem. J.*, 1941, **35**, 952) has given satisfactory results in clinical trials. Recoveries of added *p*-aminosalicylic acid were about 100 per cent. for oxalated plasma and cerebrospinal fluid, and 80 to 90 per cent. for oxalated whole blood. Streptomycin (1000 µg. per ml.) did not interfere. Sulphanilamide interferes, since the reaction is a general one for primary aromatic amines.

Reagents—(*a*) *p*-Toluene sulphonic acid solution: 20 per cent. in 0·2 *N* hydrochloric acid. (*b*) 0·75 *M* Disodium hydrogen citrate: 39·4 g. of citric acid dissolved in 188 ml. of 2 *N* sodium hydroxide and diluted to 250 ml. (*c*) Ehrlich's reagent: *p*-dimethylaminobenzaldehyde, 2 per cent. in 95 per cent. alcohol. (*d*) Sodium *p*-aminosalicylate standards: 11·4 mg. of the anhydrous salt or 13·8 mg. of the dihydrate dissolved in water and diluted to 100 ml. (equivalent to 10 mg. of *p*-aminosalicylic acid per 100 ml.).

Procedure—Dilute 0·5 ml. of blood or 1 ml. of cerebrospinal fluid (both oxalated) to 7 ml. with water, shake well, and allow to lake (3 min.). Add 3 ml. of reagent (*a*) slowly and with shaking, and filter after 5 min. To 5 ml. of the clear filtrate add 1 ml. of reagent (*b*) and 2 ml. of Ehrlich's reagent (*c*). Measure the orange colour, which develops at once and is stable for some hours, photoelectrically, using a blue filter (Ilford 602), against a blank prepared from 1·5 ml. of reagent (*a*), 1 ml. of reagent (*b*), and 2 ml. of Ehrlich's reagent, made up to 8 ml. with water. Read the *p*-aminosalicylic acid content from a calibration curve constructed by carrying out the same procedure on 1·0, 0·5, and 0·2 ml. of reagent (*d*) diluted to 7 ml.

D. C. M. ADAMSON

Water Analysis

Determination of Manganese in Natural Waters. F. Koroleff (*Acta Chemica Scandinavica*, 1947, **1**, 503–506)—Variations of 0·001 mg. of manganese per litre can be estimated by precipitating as the manganese hydroxide and using magnesium sulphate as coagulant and carrier and oxidising to permanganate with alkali periodate. Halides, organic matter, and suspended material must be removed from the water.

Method—*Reagents*—*Sodium paraperiodate*, prepared by Wells's method (*J. Amer. Chem. Soc.*, 1901, **26**, 278). *Sulphuric acid*: dilute 1 part of manganese-free concentrated acid with 2 parts of water and add 15 mg. of sodium paraperiodate per ml. *Magnesium sulphate solution*: dissolve 22·5 g. of heptahydrate in 250 ml. of water to give a solution containing 9 mg. of magnesium per ml. *Standard permanganate solution*: dissolve 57·539 mg. of recrystallised potassium permangate, add 1 ml. of 2 *N* sulphuric acid, reduce by adding 0·15 g. of sodium hydrogen sulphite, boil free from sulphur dioxide, cool, and dilute to 200 ml.; dilute 1 ml. to 60 ml., boil with 10 ml. of the sulphuric acid reagent and 2 ml. of 0·2 *N* silver nitrate, add 50 mg. of sodium paraperiodate, place in a water-bath for 30 min., cool, and dilute to 100 ml.; the solution contains 0·001 mg. of manganese per ml. and should be protected from direct sunlight.

Procedure—(*A*) *Organic matter absent*—Mix 50 ml. of 0·2 *N* potassium hydroxide with 1 litre of sea-water in a measuring cylinder. After setting aside for at least 2 hr., siphon off the supernatant liquid and dissolve the precipitate in 1·5 ml. of concentrated sulphuric acid; if this solution is coloured by organic matter proceed as in (*B*). Precipitate the halide ions with a slight excess of 2 *N* silver nitrate and filter through a Jena 3G4 porcelain filter. Boil the filtrate with 10 ml. of the sulphuric acid reagent, add 50 mg. of sodium paraperiodate, place in a water-bath for 30 min., cool, dilute to 50 ml. in a Nessler tube and compare with standards. (*B*) *Organic matter present*—Transfer the coloured sulphuric acid solution containing the manganese to a platinum dish, evaporate, and heat the residue to fusion. Cool, dissolve in 10 ml. of the sulphuric acid reagent, boil, cool, and filter through a Jena 3G4 porcelain filter. Dilute the filtrate to 75 ml., add 2 ml. of 0·2 *N* silver nitrate, and proceed as in (*A*).

C. F. HERBERT

Organic

Determination of Organic Peroxides. Evaluation of a Modified Iodimetric Method. C. D. Wagner, R. H. Smith, and E. D. Peters (*Anal. Chem.*, 1947, **19**, 976–979)—Many peroxycarboxylic acids, diacyl peroxides, hydroperoxides, and other peroxide compounds can be determined by iodimetric methods, although the accuracy of certain iodimetric methods with such simple peroxides as *cyclo*hexene and Tetralin peroxides has been disputed, especially on the grounds that the iodine liberated in the reaction disappears by addition to olefinic double bonds, and that in modifications requiring the use of strong acids there may be reduction of organic compounds other than peroxides, including any alkylene di-iodides formed by addition of iodine to olefines. Atmospheric oxygen is known to cause high results with most iodimetric methods, particularly in presence of strong acids, and methods employing sodium bicarbonate or solid carbon dioxide have been devised to overcome this defect.

For general use the method of Kokatnur and Jelling (*J. Amer. Chem. Soc.*, 1941, **63**, 1432; *Analyst*, 1941, **66**, 430), which is said to be uninfluenced by atmospheric oxygen, has been found the most suitable of the methods tested. Modifications found advantageous are (*a*) the replacement of potassium iodide by sodium iodide, which is much more soluble in the reaction mixture, thus tending to increase the reaction rate and to decrease the possible addition of iodine to unsaturated materials, and (*b*) the exclusion of water from the reaction mixture to avoid the low results obtained with auto-oxidised diolefines in its presence. In the experimental work the reaction mixture was kept in an atmosphere of carbon dioxide, and both sample and reaction mixture were de-aerated before analysis, but such precautions were found unnecessary for general use.

With the modified iodide method results with known hydroperoxides were accurate. Variation in reaction conditions produced little change in results with materials containing no auto-oxidised conjugated diolefines. Results with diolefines and ascaridole showed wide variations with reaction conditions and such bridge-type peroxides are not determined accurately by the method. Under the conditions of the method, iodine is not taken up by mono-olefines nor, in absence of peroxides, by diolefines. One experiment indicated that iodine might be absorbed by diolefines in presence of diolefine peroxides. The method with sodium iodide and *iso*propyl alcohol is as useful as that with sodium iodide and acetic acid, and has the advantages of a somewhat more general applicability and comparative freedom from interference by atmospheric oxygen. The following procedure is recommended for general use.

Procedure—Into a 250-ml. Erlenmeyer flask introduce 40 ml. of dry *iso*propyl alcohol, 2 ml. of glacial acetic acid, and up to 10 ml. (usually 5 ml.) of the sample containing not more than 2 mg.-equivalents of peroxides. Heat to refluxing temperature, add 10 ml. of *iso*propyl alcohol saturated at room temperature with sodium iodide (prepared by refluxing 25 g. of sodium iodide with 100 ml. of *iso*propyl alcohol), heat under reflux for 5 min., add 5 ml. of water, and titrate with 0·1 N or 0·01 N sodium thiosulphate. Blank determinations on the reagents will be nil, unless oxidising impurities are present in the *iso*propyl alcohol, and a blank determination on each new batch of the alcohol is sufficient. Although the method is not recommended for use with conjugated diolefines, it is sometimes useful for obtaining precise empirical results, provided that an atmosphere of carbon dioxide is maintained in the flask during the analysis.

The materials used in the investigation were tetrahydronaphthyl hydroperoxide (Tetralin peroxide) prepared by air oxidation of Tetralin at 75° C. and subsequent purification by crystallisation, α,α-dimethylbenzyl hydroperoxide (cumene peroxide), prepared from cumene by a method similar to that of Hock and Lang (*Ber.*, 1944, **77B**, 257), *tert*-butyl hydroperoxide, hydrogen peroxide (30 per cent. in water), benzoyl peroxide, and ascaridole. The following substances were auto-oxidised by allowing them to stand under oxygen in diffused daylight until analysis indicated a sufficiently high peroxide content (air was used with 2-pentene): di-*iso*butylene, 2-pentene, *cyclo*hexene, Tetralin, methylpentadiene, isoprene, and diethyl ether.

Experiments, in which known amounts of a standard solution of iodine in *iso*propyl alcohol were titrated hot in *iso*propyl alcohol containing 2 ml. of glacial acetic acid and 2 g. of sodium iodide, showed that reaction between sodium thiosulphate and the tri-iodide ion is slow unless 5 to 10 per cent. of water is present in the reaction mixture. When the titre is large, the sodium thiosulphate solution will supply enough water, but when the titre is low, water must be added before titration. Investigation of the effects of varying the manipulative details of the method showed that the amount of iodide is not critical provided that a large excess is present, that reaction time and sample size are not critical except for ascaridole, which is reduced slowly, that only the ascaridole determination was affected by the presence of water during the reaction, and that, with the exception of benzoyl peroxide, the peroxides are stable towards heat. With auto-oxidised materials variations in manipulative details had little effect on results with materials containing no conjugated diolefines, whereas results with auto-oxidised isoprene and methylpentadiene were critically dependent upon the experimental conditions. The diolefine peroxides react slowly and incompletely like ascaridole and the rate of reduction depends upon the concentration of the iodide ion in the mixture rather than upon the concentration of the peroxides. Water present during the heating under reflux sometimes causes lower results and its exclusion at this stage is recommended. The results demonstrate conclusively that peroxides in auto-oxidised conjugated diolefines differ radically from those in other materials; the latter are now generally accepted to be hydroperoxides, whereas Bodendorff (*Arch. Pharm.*, 1933, **271**, 1) has shown that conjugated diolefines form peroxides largely by 1 : 4-addition to produce intramolecular peroxides of the ascaridole type or polymeric peroxides by intermolecular peroxy-bridging to the 1- or 4-positions of other molecules.

In absence of iodide ion a large portion of the iodine is lost, particularly in presence of water. The fact that no loss of iodine occurs in presence of iodide is undoubtedly due to the formation of unreactive tri-iodide ion and the resultant small concentration of free iodine. In an examination of the application of the method to isoprene in presence of benzoyl peroxide, no peroxide-catalysed addition of iodine was observed. Some evidence was obtained of the possible absorption of iodine (as tri-iodide) by diolefines in presence of diolefine peroxides. In presence of diolefine peroxides, simple alkyl iodides are not reduced by the iodide reagent. Exclusion of air is essential only if the method is applied to diolefine samples of low peroxide content.

The method was compared with two methods in which the solvent is acetic acid. In the reflux method, 50 ml. of glacial acetic acid and 5 ml. of sample were subjected to a stream of carbon dioxide for 2 min. and the mixture was then heated to refluxing temperature. After the addition of 2 ml. of saturated aqueous sodium iodide solution, the mixture was heated under reflux for 15 min., the passing of carbon dioxide was resumed, 100 ml. of water were added, and the cooled mixture was titrated at room temperature with sodium thiosulphate to the disappearance of the yellow colour, the carbon dioxide stream being maintained during the titration. The result was corrected by means of a blank determination. The other method follows the same procedure except that after carbon dioxide has been passed through the mixture, sodium iodide solution is added, the carbon dioxide supply is discontinued, and the stoppered flask is set aside in the dark for 15 min. at room temperature before resuming the carbon dioxide flow, adding water and titrating as before. Acetic acid as a solvent offered no advantages over *iso*propyl alcohol other than the elimination of refluxing. Disadvantages are greater interference by air, occasional slight destruction of peroxides, the need for great dilution with water before titration, and increased tendency for diolefinic materials to polymerise to dark-coloured products. Use of a strong acid in place of acetic acid would probably accentuate these difficulties.

A. O. Jones

Determination of Organic Peroxides. Evaluation of the Ferrous Thiocyanate Colorimetric Method. C. D. Wagner, H. L. Clever, and E. D. Peters (*Anal. Chem.*, 1947, **19**, 980–982)—The colorimetric ferrous thiocyanate method of Young *et al.* (*Ind. Eng. Chem.*, *Anal. Ed.*, 1936, 8, 198; *Analyst*, 1936, **61**, 502) has been tested with a variety of auto-oxidised materials and is recommended as the best for frequent analyses of materials containing only small amounts of peroxides. For materials (except diolefines) containing large amounts of peroxides, the iodimetric method is more precise and accurate. The colorimetric method is not well suited for materials containing polymers, particularly diolefines, owing to the turbidity caused by insoluble high polymers.

To prepare the ferrous thiocyanate solution, dissolve 1 g. of pure ammonium thiocyanate and 1 ml. of 25 per cent. w/w sulphuric acid in 200 ml. of de-aerated methanol and shake the resulting solution with 0·2 g. of finely powdered ferrous ammonium sulphate. Place the decanted reagent (prepared fresh daily) in a brown glass-stoppered bottle.

Procedure—Place 1 ml. of the sample, preferably containing from 0·0001 to 0·0007 mg.-equivalents of reactive peroxide, in a 25-ml. volumetric flask. If necessary use 1 ml. of a suitable dilution of the sample with methanol. Dilute to the mark with ferrous thiocyanate reagent, mix well, and fill a 1-cm. cell of the Spekker photo-electric absorptiometer, equipped with blue No. 6 and green No. 5 filters, with the mixture. At 10 min. from the time of mixing, read the optical density and convert the value, corrected by a blank determination, into terms of concentration by means of a calibration curve prepared with corresponding known concentrations of ferric chloride.

In the study of the method the materials used were those listed in the preceding abstract, and these were analysed by the colorimetric method and also by the method described in that abstract. With most of the materials agreement was satisfactory, but was poor with ether and methylpentadiene (a conjugated diolefine), and with the latter substance the reduction was slow and incomplete by both methods, the discordant results being thus only of empirical value. Benzoyl peroxide also reacted only slowly with the reagent. Since good results (considering the low precision of the colorimetric method) were obtained with the pure hydroperoxides, the colorimetric method is probably fundamentally accurate when applied to auto-oxidised materials containing only isolated double bonds.

The hydroperoxides react quickly, the optical density increases rapidly during the first 2 min., and thereafter remains constant. However, the peroxides in methylpentadiene, which, presumably, are of the bridge-type described by Bodendorff (see the preceding abstract), react very slowly. Sample size does not affect results appreciably with any of the materials tested. The absence of an effect of sample size with methylpentadiene is noteworthy in view of the pronounced effect of this variable on results with conjugated diolefines by the sodium iodide method.

To study the effect of oxygen concentration, dissolved oxygen was removed from the sample and reagent before mixing by means of oxygen-free nitrogen in an apparatus similar to that of Lea (*J. Soc. Chem. Ind.*, 1945, **64**, 106; *Analyst*, 1945, **70**, 308). Lea's observation that much lower results are obtained in absence of oxygen was confirmed. When air was added after mixing the de-aerated sample and reagent, no deepening of colour occurred. This shows that the peroxide (non-diolefine) is completely and rapidly destroyed in presence of ferrous ion by both reduction and decomposition. In presence of molecular oxygen the reduction reaction proceeds much more rapidly than the decomposition reaction; in absence of oxygen the reverse is true, and only a small amount of ferrous thiocyanate is formed. Use of oxygen in place of nitrogen gives approximately normal values, thus verifying that a certain amount of oxygen is necessary to obtain quantitative results and that the effect is not one of oxidation of ferrous ion by molecular oxygen. In absence of oxygen lower values are also obtained with methyl pentadiene, although the lowering is less marked. The presence or absence of oxygen seems to have no influence on the rate of reduction of those peroxides remaining after mixing, since the optical density after mixing increases slowly and at about the same rate whether oxygen is present or not. This suggests that the hydroperoxides are destroyed rapidly upon mixing reagent and sample, leaving only the bridge-type peroxides intact.

A. O. Jones

Determination of Organic Peroxides. Evaluation of the Ferrous-Titanous Method. C. D. Wagner, R. H. Smith, and E. D. Peters (*Anal. Chem.*, 1947, **19**, 982–984)—Yule and Wilson (*Ind. Eng. Chem.*, 1931, **23**, 1254) reported that peroxides in gasolines could be quickly and conveniently determined by reducing them with ferrous thiocyanate in aqueous acetone and titrating the resulting ferric thiocyanate with standard titanous solution. Although recognising that the results did not represent actual peroxide content, they used them chiefly as a measure of the gum-forming tendencies of the gasoline. Their choice of the ferrous-titanous method rather than of iodimetric methods was based on its greater sensitivity compared with that of the Marks and Morell method (*Analyst*, 1929, **54**, 503), with which high and variable "blanks" are obtained.

The sodium iodide - *iso*propyl method and the ferrous thiocyanate colorimetric method (see the two preceding abstracts) are both basically accurate for the determination of peroxide in auto-oxidised materials that contain no conjugated diolefines. Both methods give only empirical results with conjugated diolefine samples owing to the slow rate at which such peroxides are reduced and possibly owing to side reactions. Since the method of Yule and Wilson (*loc. cit.*) is widely used particularly in the petroleum industry, its fundamental accuracy for determining peroxides in auto-oxidised materials was investigated. The prescribed correction factors, being admittedly arbitrary, were not applied.

Procedure—To prepare the ferrous thiocyanate solution dissolve 5 g. of ferrous sulphate and 5 g. of ammonium thiocyanate in 500 ml. of water and add 500 ml. of acetone. Add about 1 g. of pure iron wire and 5 ml. of concentrated sulphuric acid, and expel air by passing hydrogen or carbon dioxide through the solution. Store the product in an all-glass system under hydrogen and use it when the red colour has disappeared.

To prepare 0·01 *N* titanous sulphate, heat a mixture of 20 ml. of concentrated sulphuric acid and 80 ml. of water to 70° C. and add in small portions 0·6 g. of titanium hydride powder (Metal Hydrides, Inc., Beverly, Mass.; trade name "Altertan"). When the reaction subsides, boil the liquid on a hot-plate for 2 min., then pour it into about 900 ml. of water, freshly de-aerated with carbon dioxide. After the undissolved matter has settled, siphon the supernatant liquid into a dark storage bottle previously filled with carbon dioxide. Store the solution under hydrogen, dispense from an all-glass system and standardise daily against standard ferric chloride solution in presence of thiocyanate ions.

Measure 50 ml. of ferrous thiocyanate solution into a 250-ml. glass-stoppered Erlenmeyer flask and discharge any red colour with the least amount of titanous sulphate solution. Adjust the temperature to 25° ± 2° C., add the sample containing up to 5 mg.-equivalents of peroxide, stopper the flask, shake vigorously for 5 min. ± 5 sec., and titrate with 0·01 *N* titanous sulphate to the disappearance of the red colour.

The materials tested were as before (see the two preceding abstracts) and the method was applied to several of the known peroxides to check its accuracy. With hydroperoxides the results were far below the theoretical values or below those of the sodium iodide and *iso*propyl alcohol method. Of the peroxides tested only Tetralin peroxide and cumene peroxide were formed by natural auto-oxidation, but the results obtained with these showed that the ferrous-titanous method is inaccurate with even the simplest peroxides, whereas the iodimetric method of the preceding abstract gives satisfactory results. The fact that the results of the ferrous-titanous method are dependent upon sample size indicates, however, that it may be possible to obtain theoretical results on samples containing only very small amounts of peroxides.

Results obtained with non-diolefinic samples showed considerable similarity to those obtained with pure hydroperoxides, as might be expected. Diolefine peroxides react slowly with ferrous ions as well as with the iodide ion, the results with both methods being purely empirical. Ascaridole, a pure monomeric, 1 : 4-bridge-type peroxide, behaves in the same way, reacting slowly and incompletely with both iodide ion and ferrous ion.

In order to test the oxygen effect noted by Lea (*J. Soc. Chem. Ind.*, 1945, **64**, 106; *Analyst*, 1945, **70**, 308), experiments were made in which carbon dioxide or oxygen was passed through the reagent for 2 min. before adding the sample and shaking the mixture, and also during the titration. The results, compared with those obtained with an air atmosphere, showed a very evident dependence upon oxygen concentration, but, although those obtained with oxygen were higher in some instances than those obtained when carbon dioxide or air was used as the purging gas, they were considerably below the theoretical values. When an auto-oxidised non-diolefine has been reduced under carbon dioxide, titrated, and then air or oxygen is passed into the mixture, no further coloration appears. This indicates that the peroxides have been entirely destroyed, although they have been reduced only partly by the ferrous ion. Apparently oxygen either catalyses the reduction or inhibits the decomposition reaction. If it is assumed that the concentration of oxygen necessary for quantitative reduction of the peroxide depends upon the peroxide concentration, the low results obtained are understandable, since the ratio of dissolved oxygen to peroxide in the ferrous-titanous method is only one-hundreth of that in the colorimetric method.

The ferrous-titanous method thus appears to be empirical and useful only if values obtained by it are reproducible and can be correlated with some significant property of the test material, *e.g.*, explosion hazard or gum-forming tendency. To test the dependence of precision on the experimental conditions of the method, various materials were analysed with variations in the time of shaking, sample size, and reaction temperature. Peroxides in conjugated dienes react slowly and results are affected by sample size, temperature, and oxygen concentration. Peroxides in the other materials react almost instantaneously and results depend only upon oxygen concentration and, to a less extent, upon sample size. These results are consistent with the fact that peroxides in mono-olefines and probably those in ethers are the rapidly reacting alkyl hydroperoxides, whereas those in conjugated diolefines are the slowly reacting bridge-type peroxides. With the latter, precise results are obtainable only if the variables of the procedure are closely controlled. Benzoyl peroxide is a special instance reacting fairly rapidly with iodine ion but only slowly with ferrous ion. Ascaridole, although it is a typical diolefine peroxide monomer, seems to react rapidly in contrast with auto-oxidised diolefines. The slowly reacting diolefine peroxides may be the polymeric peroxide types mentioned by Bodendorff (*vide supra*) rather than the monomeric bridge-type forms. [Note—Tanner and Brown (*J. Inst. Petrol.*, 1946, **32**, 341; *Analyst*, 1946, **71**, 444) report a method, accurate to within ±4 per cent., which is based on the oxidation of ferrous salts, avoids the tedium of titanous chloride titrations, and needs no special colorimetric apparatus.] A. O. Jones

Direct Titration of Mercaptans in Gasoline. G. E. Mapstone (*Australian Chem. Inst. J. Proc.*, 1948, **15**, 236–241)—An alkaline nitroprusside solution is a sensitive reagent for detecting mercaptans in gasoline. Owing to the insolubility of silver mercaptides, the reaction is reversed and the colour discharged on adding silver nitrate. The reaction is made the basis of a method for the direct titration of mercaptans in gasoline. As the indicator is alkaline, addition of ammonia or some other complex-forming substance (*e.g.*, pyridine) is necessary to prevent precipitation of silver oxide. Silver mercaptides are practically insoluble even in 15 N aqueous ammonia.

The intense violet colour formed with nitroprusside rapidly changes on standing to pale green by oxidation of the nitroso group with some further breakdown to Prussian blue. To retard this irreversible reaction, it is necessary to keep the alkali concentration as low as possible. Mercaptans have a low water-solubility and are only very slightly dissociated, so that it is necessary to add substances such as amyl or butyl alcohol to maintain a significant mercaptan concentration in the aqueous phase.

Method—To 100 ml. (or other suitable volume) of the gasoline, freed, if necessary, from hydrogen sulphide by washing with 5 per cent. cadmium sulphate solution, in a 250-ml. flask, add 1 ml. each of pyridine, sodium cresylate solution (prepared by dissolving 15 ml. of re-distilled cresylic acid, free from hydrogen sulphide and mercaptans, in 85 ml. of 10 per cent. sodium hydroxide solution), and freshly prepared 0·5 per cent. sodium nitroprusside solution and then 10 ml. of amyl or butyl alcohol. Shake well and titrate with 0·05 N silver nitrate until the purple colour of the lower layer is discharged. Add a further 0·5 to 1 ml. of the nitroprusside solution, and, if the colour reappears, continue the titration, repeating the procedure until addition of more nitroprusside fails to regenerate the colour. If much silver mercaptide is precipitated during the titration, it can be coagulated by adding a pinch of fuller's earth. The percentage of mercaptan sulphur by weight is given by $3{\cdot}2\,NV/vs$, where N is the normality of the silver nitrate solution, V is the number of millilitres used in the titration, v is the volume of the sample, and s is its specific gravity. The pyridine and sodium cresylate solution may be mixed before use. The pyridine concentration is not important, but enough sodium cresylate must be added to maintain the alkalinity of the aqueous-alcoholic phase and to neutralise the acid liberated by precipitation of silver mercaptide. A suitable reagent can be prepared by adding pyridine to the sodium cresylate solution until it is cloudy and then adding enough cresylate or cresol to dissolve the excess of pyridine. Two to 4 ml. of this reagent are used in each titration.

With raw gasoline samples containing tar bases, 10 per cent. sodium hydroxide solution can be substituted for the sodium cresylate and 2 N aqueous ammonia for the pyridine. With dyed and discoloured gasolines, the colour remains in the upper layer and does not interfere with the end-point in the lower phase. With highly oxidised raw gasoline, a faint purplish colour remains, but with a little practice this can be recognised and does not then interfere.

The method compares favourably in accuracy and reproducibility with the method of Borgstrom and Reid (*Ind. Eng. Chem.*, *Anal. Ed.*, 1929, **1**, 186; *Analyst*, 1929, **54**, 767) and has the advantage that tar bases do not interfere and need not be removed.

A. O. JONES

Analysis of *Sulphite Waste Liquor and Lignosulphonates. Determination of Neutralised Solids, and Wet Oxidation for the Determination of Total Sulphur and Cations. J. R. Salvesen and D. Hogan (*Anal. Chem.*, 1948, **20**, 909–911)—*Neutralised solids*—Pipette 10 ml. of sample into 125 ml. of water, and titrate with 0·2 N sodium hydroxide to pH 8·5 to 9·0, using a glass electrode (x ml.). Weigh a weighing bottle, having a ground-glass stopper, add 10 ml. of sample, and re-weigh. Add x ml. of 0·2 N sodium hydroxide, evaporate, dry the residue at 105° C. (usually for 48 to 72 hr.), cool in a desiccator, and weigh. Correct the weight of the residue for the weight of alkali added, and so obtain the total solids. The method gives results that are reproducible to within 0·2 g. per litre, and it minimises losses of sulphur dioxide and organic acids on drying.

Total sulphur and cations—Transfer 10 ml. of sample (or 1 to 2 g. of dried sulphite liquor) into a Kjeldahl flask and rinse the neck of the flask with 10 to 15 ml. of water; add 10 to 15 ml. of concentrated nitric acid and 5 ml. of perchloric acid. It is important that sufficient nitric acid should be present to prevent an explosion. A sudden darkening of the solution indicates insufficient nitric acid. Place the flask on a steam-bath until red-brown fumes are no longer apparent, heat gently over a burner until the solution is colourless and dense white fumes appear, add 5 ml. of concentrated hydrochloric acid to the cool solution, and again heat until white fumes appear. Allow to cool, add 60 ml. of water, and heat to dissolve the salts present. Filter off and determine any silica present in the usual way, precipitate the iron and aluminium present in the filtrate as hydroxides with aqueous ammonia solution, wash the precipitate with hot 1 per cent. ammonium chloride solution, and finally dilute the filtrate to exactly 250 ml. Pipette out 100 ml. of the filtrate, make just acid to methyl orange with dilute hydrochloric acid, and precipitate the sulphate present with barium chloride. The remaining 150 ml. of the filtrate are used for the determination of calcium (as oxalate) and magnesium (by the bromometric 8-hydroxyquinoline method). The uranyl acetate method is used for the determination of sodium in the filtrate from the silica determination. The method gives results at least as precise as those obtained by the Carius method; and losses of sulphur dioxide from the sample are minimised. No hazard from the perchloric acid was experienced with sulphite waste liquors, but precautions should be taken when applying the method to other types of samples.

J. Grant

Analysis of Primary Cellulosic Materials used in Paper-making. A. Roudier (*Chimie Analyt.*, 1948, **30**, 244–249)—Notes are given on the effects of variations in the state of subdivision of the sample and in the working details of sampling and of determinations of the moisture content, ash, water- and solvent-soluble constituents, and cellulose content of pulping woods, with special reference to the French standard methods. The moisture content is determined by pre-drying the sample at 40° to 50° C. overnight and then drying at 100° C. for 5 hr.; this gives higher results than immediate drying at 100° C.

J. Grant

Analysis of the Primary Cellulosic Materials used in Paper-making. A. Roudier (*Chimie Analyt.*, 1948, **30**, 276–280)—Of the known methods for the determination of secondary polysaccharides ("hemi-celluloses"), that of Kullgren and Tyden (*Ing. Vetensk. Acad. Handl.*, Stockholm, 1929, Vol. 94), in which the furfural is oxidised to pyromucic acid by 0·05 *N* bromide - bromate (the excess of which is determined by titrating the iodine liberated from potassium iodide with 0·05 *N* sodium thiosulphate), gives good results. The acidity during the bromate titration should be 0·2 *N*, and the time of oxidation must be exactly 5 min.; the ammonium molybdate catalyst recommended by some workers is useless, or even harmful. The method of Unger and Jäger (*Ber.*, 1903, **36**, 1222), in which barbituric acid is used to precipitate the furfural, gives good results, but is inconvenient because the reagent must be recrystallised from water, its 1 per cent. solution in 12 per cent. hydrochloric acid is unstable, and the precipitate is difficult to filter. The only drawback of the Simon method (*Biochem. Z.*, 1932, **247**, 171), in which the furfural is precipitated by 2 : 4-dinitrophenylhydrazine, is that it is a gravimetric method. The colorimetric aniline acetate method (Duffau, *Bull. Soc. Chim. biol.*, 1946, **28**, 873) is very sensitive for small quantities, but its accuracy (±5 per cent.) is less than that of the three other methods.

J. Grant

Inorganic

New Method for the Determination of Water. M. Pesez (*Bull. Soc. Chim.*, 1948, 1108–1109M)—Lead tetra-acetate is decomposed by water, with the formation of lead dioxide, thus—

$$(CH_3COO)_4Pb + 2H_2O \rightarrow PbO_2 + 4CH_3COOH$$

Iodimetric determination of the lead dioxide then affords a measure of the quantity of water originally present. The method is suitable for the examination of solvents.

Lead tetra-acetate reagent—Shake 20 g. of minium with 80 ml. of acetic acid (m.p. 16° to 16·6° C.) for 15 min. at 40° to 50° C., and then heat at 70° to 80° C. until dissolution is complete. Cool quickly, and filter off the crystallised lead tetra-acetate, wash it with 50 ml. of acetic acid and then with 100 ml. of light petroleum, without allowing it to become dry. Dry *in vacuo* at 40° C. and store out of contact with air and moisture. Dissolve 3 g. of the tetra-acetate in 100 ml. of dry benzene in a stoppered flask. Some lead dioxide is formed through the hydrolytic action of residual traces of water in the crystals. This is removed by sedimentation or by centrifuging.

Aceto-iodide reagent—To 50 ml. of acetic acid add, slowly with cooling, 50 ml. of sodium hydroxide solution ($d = 1{\cdot}4$) and then 7·5 g. of potassium iodide dissolved in 50 ml. of water. Store in the dark.

Detection of water—To 5 ml. of the tetra-acetate reagent add 1 to 2 ml. of the liquid for test. If water is present a yellow-brown colour and then a brown precipitate of lead dioxide, are formed. The sensitivity is 1 in 200,000 when testing non-hydroxylic solvents. With methyl and ethyl alcohols the sensitivity is of the same order, but the lead dioxide does not precipitate. The colour generated can be used for the estimation of traces of water in these alcohols by comparison with standards.

Determination of water—Place 5 ml. of the liquid for test, or 5 ml. of a suitable dilution in benzene, in a stoppered centrifuge tube. Add 10 ml. of the tetra-acetate, allow to stand for 30 min. in the dark, and then centrifuge. Confirm the presence of an excess of lead tetra-acetate in the supernatant liquid by adding it to 5 ml. of acetone containing 1 per cent. of water. Suspend the centrifuged lead dioxide in 5 ml. of benzene, centrifuge, and decant. Suspend the dioxide in 1 ml. of acetone, dissolve in 20 ml. of aceto-iodide reagent, and titrate with 0·02 *N* sodium thiosulphate. 1 ml. of 0·02 *N* sodium thiosulphate is equivalent to 0·00036 g. of water.

The operations must be carried out rapidly with special care for the exclusion of moisture.

W. C. Johnson

Colour Reaction of the Alkaline Earths. A. Okáč and J. Pech (*Coll. Czech. Chem. Comm.*, 1948, **13**, 400–406)—In alkaline solution the alkaline earths give an intense violet colour, or at higher concentrations a blue precipitate, with pyrogallol carboxylic acid, which can be prepared by the method of Thiele and Jaeger (*Ber.*, 1901, **34**, 2842) or Konstanecki (*Ber.*, 1885, **18**, 3205).

The neutral test solution is made alkaline with sodium hydroxide and filtered if a precipitate forms, and a freshly-prepared, saturated, aqueous solution of the reagent is added.

The reaction is inhibited by strong reducing agents capable of energetic action in alkaline media (*e.g.*, stannites), and by anions such as phosphate, fluoride, oxalate, and carbonate which reduce the cation concentration in the solution. The solubilities of the alkaline-earth sulphates are such that addition of sulphate lowers the concentration of barium and strontium below the sensitivity of the reaction for these cations, and calcium may be detected in a mixture of the three by acidifying the test solution with sulphuric acid, boiling, rendering the solution alkaline, and adding an aqueous solution of the reagent. Filtration of the acid solution is advisable as the colour is adsorbed on the precipitate.

In general, the hydroxides of other metals should be precipitated and filtered off; the use of tartrate or cyanide to limit precipitation renders the colour produced by the alkaline earths unstable. The permanence of the colour depends on pH, reagent concentration, and atmospheric oxidation of the solution. The use of ammonia instead of sodium hydroxide gives a finer colour, but the reaction is less sensitive and the colour less permanent.

Procedure—On a micro scale, treat 0·1 ml. of solution in a micro test tube with 1 drop of 2 *N* sodium hydroxide and 2 drops of a 1 per cent. ether solution of the reagent. Remove any precipitate produced by the alkali by centrifuging before adding the reagent. In this volume the reaction is sensitive to 1 part of barium in 10,000, 1 part of strontium in 20,000, and 1 part of calcium in 200,000. The test is not suitable for use on a drop-reaction plate, or on drop-reaction paper, as the large surface exposed leads to immediate oxidation of the alkaline reagent to a dark brown solution. M. E. DALZIEL

Polarographic Copper, Zinc, and Manganese Determinations in Aluminium and Zinc Alloys. M. Spálenka (*Z. anal. Chem.*, 1947, **128**, 42–51)—*Procedure for the determination of copper in aluminium or zinc alloys and of zinc in aluminium alloys containing little copper*—Warm 0·3 g. of alloy with 10 ml. of 5·0 *N* hydrochloric acid and dissolve any residue by adding 1 drop of concentrated nitric acid. Dilute to approximately 50 ml. with water and add 25 ml. of 10 *N* aqueous ammonia. Cool to room temperature, transfer to a 100-ml. volumetric flask and, after adding 1 ml. of saturated sodium sulphite solution and 1 ml. of gelatin solution (0·5 g. of gelatin and 3 ml. of concentrated hydrochloric acid in 100 ml.), dilute to 100 ml. with water. Record a polarogram from 0 to −0·6 v. for copper determinations, or from −0·8 to −1·4 v. for zinc determinations.

This procedure can be used for alloys containing between 0·03 and 10 per cent. of copper or between 0·1 and 15 per cent. of zinc.

Procedure for the determination of zinc in aluminium alloys that are rich in copper—Dissolve 0·5 g. of alloy, containing between 0·007 and 4·0 per cent. of zinc, in 10 ml. of 3·5 *N* sodium hydroxide, dilute with 10 ml. of water, cool, and add 15 ml. of the ammonium chloride solution (4·5 g. of ammonium chloride and 350 ml. of concentrated aqueous ammonia solution in 1 litre), 1 ml. of saturated sodium sulphate solution, and 2 ml. of gelatin solution. Dilute to 500 ml., and examine a portion of the solution polarographically between −0·6 and −1·6 v.

Procedure for determining manganese in aluminium alloys—Warm 0·5 g. of alloy with 8 ml. of 5·0 *N* hydrochloric acid until all the aluminium has dissolved. Heat to boiling and then add sufficient 30 per cent. hydrogen peroxide (5 to 20 drops) to dissolve the copper. Cool slightly and add 2 ml. of saturated sodium sulphite solution and 3 ml. of Metol solution (0·1 g. of Metol, 2 ml. of a saturated solution of sodium sulphite, and 5 ml. of concentrated hydrochloric acid in 100 ml.), stirring well after each addition. Add 15 ml. of tartrate solution (500 g. of Rochelle salt and 10 ml. of a saturated solution of sodium sulphite in 1 litre) and 10 ml. of 2·5 *M* potassium cyanide solution (164 g. of potassium cyanide, 200 g. of sodium hydroxide, and 10 ml. of a saturated solution of sodium sulphate in 1 litre), stir, and cool to room temperature. Add 1 ml. of gelatin solution, dilute to 50 ml. with water, and examine the solution polarographically from 0 to −1·2 v.

Procedure for determining manganese in zinc alloys—Follow the procedure for determining manganese in aluminium alloys as far as the addition of the Metol solution. Then add 10 ml. of aqueous ammonia solution containing sodium sulphite (500 ml. of concentrated aqueous ammonia solution and 20 ml. of a saturated solution of sodium sulphite in 1 litre), stir and, after cooling to room temperature, add 15 ml. of 5·0 *M* potassium cyanide solution (326 g. of potassium cyanide and 20 g. of sodium hydroxide in 1 litre), 5 ml. of 10 *N* sodium hydroxide solution containing 5 per cent. by volume of a saturated solution of sodium sulphite, and 1 ml. of gelatin solution. Dilute to 50 ml. with water and examine a portion of the solution polarographically between 0 and −1·2 v.

The last two procedures can be used for alloys containing between 0·04 and 4·0 per cent. of manganese.

The copper, manganese, or zinc concentrations are calculated by adding a known amount of a standard alloy to the sample under examination, and comparing the height of the step given by the sample alone with that of the step given by the sample plus standard alloy. J. G. WALLER

Masking of Molybdenum, Tungsten, and Vanadium Reactions by Fluoride. F. Feigl (*Analyt. Chim. Acta*, 1948, **2**, 397–401)—The reactions of molybdate with silver nitrate, potassium ferrocyanide, potassium thiocyanate under reducing conditions, hydrogen peroxide, 8-hydroxyquinoline, rhodamine B, alkali xanthogenate, or benzoinoxime do not occur in presence of sufficient amounts of fluoride. Similarly, tungstates give no reaction with mineral acids, silver nitrate, stannous chloride, zinc and hydrochloric acid, benzidine, diphenyline, cinchonine, 8-hydroxyquinoline, or rhodamine B if sufficient fluoride is present. Fluoride also masks the reaction of vanadate with zinc and hydrochloric acid, silver nitrate, or hydrogen peroxide, but does not affect the reaction with cupferron; potassium ferrocyanide gives a yellow precipitate, benzidine a violet precipitate, and 8-hydroxyquinoline a blue-green colour in the presence of fluoride, as against a green precipitate, a green precipitate, and a brown precipitate, respectively, in its absence.

The reason for these effects lies in the formation of complex "fluorised" molybdic, tungstic, and vanadic acids; this depends on equilibrium reactions. Treatment with boric acid has a "demasking effect" by removing fluoride as hydrofluoboric acid.

These effects can be demonstrated by treating a solution of molybdate in acetic acid with an excess of silver nitrate and dissolving the precipitate by adding potassium hydrogen fluoride. Treat a third of the solution with saturated boric acid solution, and the precipitate reappears; add 8-hydroxyquinoline acetate to another third, and a yellow oxine precipitate appears; add potassium hydrogen fluoride and 8-hydroxyquinoline and no precipitate appears, proving that the masking effect depends on the quantity of the masking ion.

M. E. DALZIEL

Colorimetric Determination of Traces of Gold. E. B. Sandell (*Anal. Chem.*, 1948, **20**, 253–256)—Quantities of gold from 0·1 to 10 μg are isolated by precipitation with stannous chloride, with tellurium as collector, and estimated colorimetrically or photometrically with *p*-diethylaminobenzylidenerhodanine as reagent. A colloidal solution of a red-violet compound is formed by adding an alcoholic solution of the reagent to a weakly acid, auric gold solution. The sensitivity of the reaction decreases as the mineral acid concentration is increased, but the reproducibility of results is better with 0·12 *M* hydrochloric acid than with a 0·075 *M* solution.

METHOD — *Apparatus* — Several flat-bottomed, glass-stoppered tubes, about 1·2 × 8 cm. and graduated at 5 ml. or 4·5 ml.

For the filtration, use a small, wide-mouthed bell-jar resting on a piece of ground-glass and fitted with a two-hole rubber stopper carrying a small funnel. Use a rubber ring to fit the filter-crucible into the funnel.

Reagents — *p-Diethylaminobenzylidenerhodanine solution*, containing 0·05 g. in 100 ml. of absolute ethyl alcohol. *Stannous chloride solution*—Dissolve 20 g. of the dihydrate in 100 ml. of 2 *N* hydrochloric acid. *Tellurium tetrachloride solution*—Add 1 to 2 ml. of nitric acid to 100 mg. of precipitated tellurium and evaporate to dryness. Dissolve the residue in 10 ml. of concentrated hydrochloric acid and dilute to 100 ml. *Standard gold solution*—Prepare a solution containing 0·001 per cent. of gold as chloroauric acid in 0·10 *M* hydrochloric acid.

Procedure—Prepare a solution of the sample containing 0·1 to 10 μg. of gold in about 50 ml., and not containing any strong oxidising agents. Make the solution 2·5 *N* with respect to hydrochloric acid and add 0·2 ml. of the tellurium solution. Add 5 ml. or more of the stannous chloride solution to produce a brown colloidal precipitate of tellurium and add an excess of 3 to 5 ml. Heat to the boiling-point and keep at this temperature for 30 min. to coagulate the precipitate. Collect the precipitate in a 7-ml. porous, porcelain filter-crucible and wash the beaker and filter with five 5-ml. portions of diluted hydrochloric acid (1 + 4). Add 1 ml. of *aqua regia* to the beaker used for the precipitation, heat the contents almost to boiling-point, and pour into the crucible. Bring the acid into contact with the tellurium on the walls of the crucible and when all or most of the precipitate has dissolved, draw the solution through the crucible into a 20-ml. Pyrex beaker. Repeat the operation with a further 1 ml. of *aqua regia*. Wash the beaker and crucible with two small portions of water. Evaporate the solution to dryness, avoiding prolonged heating of the residue. Add 0·01 ml. of *aqua regia* to the cold residue and with the aid of a stirring rod moisten the walls of the beaker with the acid. Allow the acid to evaporate at room temperature.

Prepare standards containing 0, 0·2, and 0·4 μg. of gold by transferring quantities of standard gold solution to the glass-stoppered tubes, adding 0·3 ml. of 2·0 *M* hydrochloric acid, and diluting with re-distilled water to nearly 4 ml. Add 0·25 ml. of 1 per cent. sodium fluoride solution and mix.

Add 0·3 ml. of 2·0 *M* hydrochloric acid to the dry tellurium residue, moistening all the residue with the acid. Add 1 ml. of water and stir. If the solution is clear, transfer to a tube and rinse the beaker with re-distilled water to bring the volume in the tube to 3·5 ml. If the solution is turbid, filter through a small, porcelain filter-crucible into a tube and wash with small portions of water to bring the total volume to 3·5 ml.

Add 0·25 ml. of 1 per cent. sodium fluoride solution to the sample and standard tubes, and mix. Add 0·3 ml. of the rhodanine reagent to each tube and mix by inverting three times. Dilute to the mark and mix by inverting three times. After a few minutes, compare the colour of the sample with the standards and if it is less strongly coloured than the 0·4-μg. standard, find the gold content by visual comparison. Otherwise, transfer the sample solution to a dry absorption cell and, using a green filter, measure the extinction exactly 10 min., or other fixed time, after the addition of the rhodanine reagent. To obtain the standard extinction curve take 0, 0·5, 2·5, 5, 7·5, and 10 μg. of gold, add 2·0 *M* hydrochloric acid to make the total volume 0·3 ml., treat with sodium fluoride solution and rhodanine reagent as described above, and dilute to volume. A graph of extinction against concentration should be a straight line. Extinction measurements should be reproducible to within 0·001.

Silver, iron, copper, lead, and arsenic do not interfere, and small amounts of mercury, thallium, platinum, molybdenum, titanium, tungsten, uranium, vanadium, zirconium, bismuth, and germanium are without effect. Palladium reacts with the rhodanine reagent. Up to 3 μg. of palladium in the final volume of 5 ml. can be made harmless by adding 0·05 ml. of a 1 per cent. alcoholic solution of dimethylglyoxime to the acid sample solution before the addition of the rhodanine reagent. For the estimation of gold in biological material the procedure must be modified to ensure the removal of carbon.

Procedure for biological material—Ash the sample in a porcelain dish at low red heat. Transfer 1 to 2 g. of the ash to a small porcelain dish, add a few millilitres of water, followed by 2 ml. of nitric acid added in portions. Evaporate to dryness and heat gradually to dull red heat. Keep at this temperature for about 15 min., or until brown fumes cease to be evolved. Add 5 ml. of diluted hydrochloric acid (1 + 1) and evaporate to dryness. Add another 5-ml. portion of the acid and repeat the evaporation. Take up the residue in a mixture of 5 ml. of concentrated hydrochloric acid, 5 ml. of water, and 2 ml. of saturated bromine water. Heat nearly to the boiling-point, with stirring, and filter through a sintered-glass crucible. Wash with 10 ml. of diluted hydrochloric acid (1 + 1) and a few portions of water. Transfer the filtrate and washings to a 100-ml. beaker, dilute to 50 ml., and add 0·2 ml. of tellurium solution and 5 ml., or more, if required, of stannous chloride solution. Continue as in the above procedure.

B. ATKINSON

Reviews

QUALITATIVE ANALYSIS BY SPOT TESTS: INORGANIC AND ORGANIC APPLICATIONS: FRITZ FEIGL. Third English Edition, translated by RALPH E. OESPER. Pp. xvi + 574. New York and Amsterdam: Elsevier Publishing Company, Inc. London Distributors: Cleaver-Hume Press Ltd. 1947. Price 43s. net.

Everyone who has any acquaintance with modern analytical methods is familiar with the name of Dr. Fritz Feigl, and with the place that he has made for himself in the inception and development of the technique and methods of analysis by the use of spot tests. Most will also be familiar with one or other of the earlier editions of this work, translated by Dr. Matthews, which appeared in 1937 and 1939.

Since the appearance of the third German edition, on which the second English edition was based, a vast mass of additional material on this branch of microchemistry has appeared in the literature, and for some time it was clear to the author that a new edition, incorporating a critical selection of the fresh material, was necessary. Owing to difficulties of communication during the war, it was necessary to choose a translator in the Western Hemisphere. Those chemists familiar with Dr. Feigl's research publications are also well acquainted with the name of Dr. Oesper in this, among other capacities.

There is much new material of value in this edition. There are detailed descriptions, not included in earlier editions, of a wide range of techniques used in spot analysis. Many new spot reactions, both inorganic and organic, are described with full working directions, whilst the text carried over from previous editions has been completely revised. The bibliography has been extended to include many additional references, and, indeed, the author, to quote his own intentions, "has tried to assemble and present the entire literature on spot test analysis in such a form that the reader will be able to secure here a rapid survey of the available spot tests and their application." Some idea of the comprehensiveness of the book can be given by noting that there is an eight-page author index in addition to the excellent 56-page subject index.

The reviewer has nothing but admiration for the contents of the book, and can only add that he considers it to be an essential part of the library of every up-to-date analytical chemist. The production—binding, printing, illustration—also is excellent, subject only to the criticism that the paper is hardly sufficiently opaque, so that the ghost of the printing on the reverse side can be seen. This is a trifle tiresome: it may, however, be the result of an attempt to reduce somewhat the bulk of a volume covering so comprehensively all practical aspects of a fascinating and invaluable branch of microchemistry.

CECIL L. WILSON

THE PLANT ALKALOIDS. By T. A. HENRY. Fourth Edition. Pp. xxiii + 804. London: J. and A. Churchill, Ltd. 1949. Price 63s. net.

There is a small and select number of scientific books, a new edition of which one opens with a sense of pleasurable anticipation. Henry's "Plant Alkaloids" is one of this class. It stands unchallenged as the classic work on alkaloids, and one knows in advance that the information given will be full, accurate, up-to-date and lucidly expressed. The new edition does not disappoint.

Very much has been re-written and the size has been expanded from 689 to 804 pages. A rough calculation shows that some 2,000 different alkaloids are mentioned. The labour of keeping such a book up to date must be great indeed, and Dr. Henry is to be congratulated on the care and accuracy with which it has been done. The method of arrangement and classification of the earlier edition has been maintained, though the author finds it increasingly difficult to adhere to the system of classification based on nuclear structure for many of the more complex alkaloids. As he characteristically writes, "Nature does not produce alkaloids to meet the needs of either botanical or chemical systematists."

The structural formulae, which occur in great numbers, are admirably drawn and reproduced,—a protracted search has failed to discover a single tervalent carbon atom.

From the medicinal standpoint, the vast amount of work carried out on alkaloids since the last edition of this book was published is disappointing. No new alkaloid has been found possessing outstanding pharmacological properties. The British Pharmacopoeia 1948 contains sixteen of the alkaloids described in this book, and all of these have been known for many years. Ergometrine, the most recent, was discovered in 1935. Tubocurarine, not in the B.P., has recently found a valuable use in medicine but already seems likely to be replaced by synthetic compounds. One wonders for how long alkaloids will retain a place in medicine. Cocaine is falling out of use, quinine faces powerful competition from synthetic rivals and even morphine is threatened. But old remedies die hard and many more editions of this book will surely be required before the last alkaloid is omitted from the B.P.

Interesting developments have occurred in the elucidation of the structure of alkaloids. The discovery that certain alkaloids of *Aconitum, Delphinium, Veratrum* and *Solanum* species contain a tetracyclic system identical with, or closely related to, that of the steroids and that the alkaloid solanine is a glycoside, is of great interest in relation to the biogenesis of alkaloids in the plant. In fact, great interest is now being shown in the problem of biogenesis, particularly in devising methods of synthesis that might occur in the plant itself.

The book is, of course, indispensable to the drug analyst. Although no attempt is made to describe methods of analysis except in general terms, a great deal of information on methods of detection and identification is given and the comprehensive lists of references guide the reader to the original sources.

NORMAN EVERS

MICROCHEMISTRY GROUP

A JOINT Meeting of the Group with the East Midlands Section of the Royal Institute of Chemistry will be held in Nottingham on Thursday, September 22nd, 1949. In the afternoon a limited number of members will visit the laboratories of Boots Pure Drug Company at Beeston and Nottingham.

At 6.30 p.m. a meeting will be held at Nottingham Technical College, at which the following papers on Microbalances will be read and discussed:

"Microchemical Balance Design," by George F. Hodsman, B.Sc., Ph.D., A.Inst.P.
"Maintenance and Precision of Microbalances," by David W. Wilson, M.Sc., F.R.I.C.
"The Ultra Microbalance," by Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.

In an adjoining room an Exhibition of Microbalances will be on view from 5 p.m. to 9 p.m.

PHYSICAL METHODS GROUP

A MEETING of the Group, organised by its Polarographic Discussion Panel, will be held in Sheffield, on Friday, October 7th, 1949.

At 2·30 p.m. there will be a visit to the Bragg Laboratory, Naval Ordnance Inspection Department, followed by tea in the Laboratory.

At 6.30 p.m., a Joint Meeting with the Sheffield Section of the Royal Institute of Chemistry and the Sheffield University Chemical Society, on "Polarographic Analysis," will be held in the Chemical Lecture Theatre of Sheffield University. The following papers will be read:

"The Use of Polarographic Methods for the Analysis of Fine Chemicals," by G. H. Osborn, A.R.I.C.
"Applications of Mercury Drop Control to Differential and Derivative Polarography," by L. Airey, B.Sc., A.R.I.C., and A. A. Smales, B.Sc., A.R.I.C.
"Diffusion Current Measurement with the Tinsley Polarograph," by W. Furness, B.Sc., F.R.I.C.

The following further meetings of the Physical Methods Group have been arranged:

November 29th, 1949: At the Imperial College, London, S.W.7, the Annual General Meeting of the Group, followed by an address on "The Mass Spectrometer, a Survey of its Applications in Analysis," by Dr. J. G. A. Griffiths.
January 3rd, 1950: At the Imperial College, London, S.W.7, a discussion in Spectroscopic Analysis.
February 1st, 1950: In London, a discussion on Modern Methods of Moisture Determination.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Extraordinary General Meeting of the Society was held at 6 p.m. on Wednesday, August 24th, 1949, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.2. The President occupied the chair. The following Special Resolution, which had been recommended by the Council, was proposed by the President, seconded and carried unanimously.

"That the Articles of Association of the Society be altered as follows:—

"Article 29.—By deleting the article and side note and substituting therefor the following:—

> 29. Votes may be given either personally or by proxy, except in the case of voting by ballot under Article 36 (A) hereof, in which case votes may be given personally or through the post as provided by that Article. No person shall act as proxy unless he is a member of the Society." Proxies

DEATHS

We regret to record the deaths of

Sir Robert Robertson (Honorary Member).
Narenda Nath Dutta.
John William Hawley.
Clifford Hanks Robinson.
Herbert Frederick Stephenson.

The Statistical Use of Several Analytical Constituents for Calculating Proportions of Ingredients in Certain Food Products

By E. H. STEINER

The application of statistics in calculating the proportion of an ingredient in a food product has already been considered in the case where only one analytical constituent is available for the determination.[1] It was shown how limits can be assigned within which the true proportion of the ingredient is likely to lie, and that these limits depend primarily on the amount of permissible risk of rejecting a product as deficient in the ingredient when it has in fact the correct proportion. For any pre-assigned risk, however, the values of the limits depend on the natural variation encountered among the values of the proportion of the constituent in samples of the ingredient. No improvement in analytical accuracy can overcome this source of variation (and consequent error in the final estimation), but there remains the possibility of lessening this error by the use of two or more constituents in the determination of the ingredient. For example, in the analysis of fruit pulps, determinations of both soluble solids and insoluble solids might be made. It is the purpose of the present paper to show how several such measurements may best be combined to obtain the most accurate assessment of the proportion of ingredient present.

THE COMBINATION OF TWO CONSTITUENTS

Various ways of combining data from two different determinations are possible. For example, the apparent percentages of an ingredient calculated from different constituents might simply be averaged in the usual way. Suppose a strawberry pulp shows 7·0 per cent. of soluble solids (determined by specific gravity) and 0·80 per cent. of free acid (as hydrated citric acid). Average figures for these two constituents in strawberries are 9·01 and 0·96 per cent. (these and other figures quoted below are based on data collected over a large number of years at the laboratories of the British Food Manufacturing Industries Research Association.) Separate estimates of the fruit content of the pulp would then be 77·7 per cent. from the soluble solids and 83·3 per cent. from the acidity.

Averaging these two results would give 80·5 per cent. as an improved estimate of the proportion of fruit, but this is not necessarily the best that can be made. It ignores, for instance, differences in variability shown by the respective constituents in the fruit itself. Actually, soluble solids show only about half the variability of free acid in samples of strawberries (the coefficients of variation are 10·3 and 24·2 respectively), so that relatively more weight should be attached to estimates based on the former. Further, no account is taken of any correlation between the constituent values. If those samples of strawberries with high soluble solids contents tend to have high free acid values, and *vice versa,* there is obviously little to be gained from averaging the two estimates.

The possibility of improving the estimate by the use of two constituents is governed partly by the relative extent to which the constituents vary in the fruit, and also by the degree of correlation between them. If the soluble solids and free acids contents show an association or correlation as above to a high degree, then the combination "soluble solids minus free acid" would be expected to show less variation than either of them singly. Such a combination could be used as a *single* analytical figure with which to divide the corresponding quantity observed in the fruit pulp. Similarly, if the two constituents are negatively correlated, the combination "soluble solids plus free acid" should be more constant. In the example quoted above, these two combinations would lead to apparent percentages of strawberries in the pulp of $\frac{6{\cdot}2}{8{\cdot}05} \times 100 = 77{\cdot}0$ per cent. and $\frac{7{\cdot}8}{9{\cdot}97} \times 100 = 78{\cdot}2$ per cent. respectively.

The optimum combination

The practice of averaging the two apparent percentages calculated from each constituent separately is precisely equivalent to weighting constituents in inverse proportion to their mean values and using the combination as a single figure. Viewed in this way, the normal procedure and the other two suggested above are particular instances of using a certain linear combination of the constituents. Now the variation of any such linear combination can be expressed exactly in terms of the standard deviations and correlation coefficients of the components, hence it becomes possible to calculate multiplying factors for weighting the coefficients, so that the variation of the resulting combination is less than that of any other linear combination. This will be called the optimum combination.

It will be observed that the apparent proportion of fruit varies according to the particular combination of the constituents used. Clearly all three values calculated above for the percentage of strawberries cannot be correct and, in the absence of information on the precise soluble solids and free acid contents of the strawberries used for the pulp, we cannot assert that any value calculated represents the true value. We can, however, select that percentage resulting from the use of the combination that shows least variation in samples of strawberries. By the consistent use of this combination with all samples of strawberry pulp, we shall, *on average,* be nearer the truth than by the use of any other. It is in this sense that the optimum combination arrived at will be considered the best, and the resulting estimate of the apparent proportion of ingredient the most accurate one.

In considering the calculation of the proportion of an ingredient in a foodstuff from the amount of a single constituent characteristic of the ingredient,[1] it was shown that where the amount of the constituent in the foodstuff is determinable exactly, *i.e.,* without significant analytical error, the accuracy of the estimate of the amount of the ingredient depends solely on the coefficient of variation of the constituent in samples of the ingredient. This condition applies to fruit pulps, where the whole of the value of the constituent determined in the pulp is contributed by the ingredient and the experimental error is small compared with the natural variation shown by the ingredient. In such cases the maximum improvement in accuracy is obtained when the coefficient of variation is minimised, by the combined use of two or more constituents. In the more general type of analysis, as in meat products or where the analytical error is appreciable, the proportion of ingredient is calculated from the ratio of *two* variable quantities. The problem of combining measurements to provide the most accurate final analysis is then more complex, and will not be considered here, in view of the fact that suitable data for treatment appear to be lacking.

Calculation of the optimum combination of two constituents

Let x_1 and x_2 denote two different constituents characteristic of the ingredient (*e.g.*, soluble solids and free acid in strawberries). Also let their mean values and standard deviations be $\bar{x}_1$, σ_1 and $\bar{x}_2$, σ_2 respectively and r be the correlation coefficient.

Consider a combination of the two constituents given by $x_c = x_1 + kx_2$, where k is an arbitrary constant. (We could have made the more formal assumption of two constants, k_1 and k_2, corresponding to x_1 and x_2, respectively, but there is no loss of generality in putting that of x_1 equal to unity, since any constants proportional to these factors can be used without affecting the coefficient of variation of the combination, and the alternative chosen gives simpler expressions.) Then, since x_c is a linear function of x_1 and x_2, the combined quantity will have a mean $\bar{x}_c = \bar{x}_1 + k\bar{x}_2$ and standard deviation $\sigma_c = \sqrt{\sigma_1^2 + 2kr\sigma_1\sigma_2 + k^2\sigma_2^2}$. This latter expression is exact if σ_1 and σ_2 are the true values for the constituents, but in practice only a small number of observations are usually available for estimating these quantities. If s_1 and s_2 are the standard deviations estimated from a total of n samples of ingredient analysed, then s_c, the estimate of σ_c, should be regarded as based on (n − 2) degrees of freedom. The reduction in the number of degrees of freedom is reasonable if it is considered that *two* constants have really been fitted to the data in arriving at the variance of the optimum combination, one being the mean value and the other the factor k. If only two samples of the ingredient had been analysed a value of k could be found such that the resulting combination was a constant (for the two samples), but this would have no practical significance. By analogy with multiple regression theory, one degree of freedom is lost every time a fresh constant is fitted to the data. Since s_1 and s_2 are themselves calculated by dividing the sum of squares by (n − 1), the estimated standard deviation of the combination will be

$$s_c = \sqrt{(s_1^2 + 2krs_1s_2 + k^2s_2^2) \times (n-1)/(n-2)}.$$

The necessary value of k for the optimum combination is that which minimises the coefficient of variation given by $V_c = 100s_c/\bar{x}_c$. This may be found by differentiating V_c with respect to k, and equating to zero, leading to the value:

$$k = s_1(V_1 - rV_2)/s_2(V_2 - rV_1) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \qquad (1)$$

where V_1 and V_2 are the observed coefficients of variation of the two constituents.

If this value of k is inserted in the above expressions for s_c and $\bar{x}_c$, we find for the coefficient of variation of the optimum combination:

$$V_c = V_1V_2\sqrt{\frac{1 - r^2}{V_1^2 - 2rV_1V_2 + V_2^2} \times \frac{n-1}{n-2}} \quad .. \qquad (2)$$

Equations (1) and (2) enable the value of the optimum combination $x_1 + kx_2$ and its coefficient of variation to be calculated for any pair of constituents available for estimating an ingredient.

From a consideration of equation (2) it is possible to draw general conclusions on the benefit to be gained from combining constituents in this way. If the constituents are uncorrelated (r = 0) the greatest gain will occur when the two coefficients of variation are equal; in this event a reduction of about 30 per cent. in the probable range is effected. If the correlation is negative, a still greater reduction results, increasing as r approaches minus one. If the correlation is positive, the gain is greatest when the ratio of smaller to larger coefficient of variation differs most from the correlation coefficient numerically. No improvement is possible when these two quantities are equal, so that even a high positive correlation (as considered on page 430) is useless when the coefficients of variation are similar. This is obvious if the correlation coefficient is unity and the coefficients of variation equal, since one set of values is then a multiple of the other and provides no fresh information. These conclusions are borne out in Table I.

TABLE I

OPTIMUM COMBINATION OF SOLUBLE SOLIDS WITH FREE ACID (AS HYDRATED CITRIC ACID) IN SOME COMMON FRUITS

	Soluble solids		Free acid				Optimum combination		
Fruit	Mean value, % $\bar{x}_1$	Coefficient of variation V_1	Mean value, % $\bar{x}_2$	Coefficient of variation V_2	Correlation coefficient r	Number of samples n	Free acid factor k	Mean value $\bar{x}_c$	Coefficient of variation V_c
Apple ..	11·99	10·5	1·09	42·2	− 0·59	37	1·99	14·16	7·4
Blackcurrant	14·52	13·3	3·53	11·0	− 0·12	20	5·91	35·38	8·2
Gooseberry ..	8·28	9·7	2·21	14·5	+ 0·39	48	0·95	10·38	9·4
Plum	12·59	17·9	1·74	27·9	0·34	50	1·77	15·67	17·2
Raspberry ..	8·70	17·4	1·70	18·0	+ 0·17	29	4·67	16·64	13·7
Strawberry ..	9·01	10·3	0·96	24·2	+ 0·23	43	0·86	9·84	10·2

It is evident that in order to find the optimum combination of two analytical constituents it is necessary to calculate, not only their means and standard deviations, but also their correlation coefficient. This can only be done where determinations of the constituent values have been made on the *same* samples of the ingredient and imposes restrictions on the usefulness of published data in this respect. For fruits, however, many data of the required type are available and some of them have been published.[2]

Table I shows the values of k and V_c for the optimum combination of soluble solids with free acid in some common fruits. It is immaterial to which constituent it is decided to apply the factor k so long as it is remembered that s_2 and V_2 in equation (1) relate to measurements of this constituent (*i.e.*, x_2). In Table I, k is applied to the free acid constituent, the soluble solids factor being taken as unity. To obtain the most accurate estimate of fruit content in a fruit pulp from these two measurements, the free acid percentage in the pulp is multiplied by the appropriate value of k and added to the soluble solids percentage. The quantity so obtained, multiplied by 100, is then divided by the corresponding quantity for the fruit itself, (*viz.*, the mean percentage of free acid, multiplied by the same factor) and then added to the soluble solids in the fruit. Thus, for the strawberry pulp considered previously, this leads to an estimated fruit content of $\frac{(0{\cdot}8 \times 0{\cdot}86) + 7{\cdot}0}{(0{\cdot}96 \times 0{\cdot}86) + 9{\cdot}01} \times 100 = 78{\cdot}1$ per cent. Table I shows the mean values of the optimum combination for the various fruits, to use in estimating fruit contents of pulps when these two constituents are employed in analysis. They are calculated from the mean values shown for the separate constituents.

Reference to Table I shows that with some fruits, notably apple, blackcurrant and raspberry, a substantial reduction in the coefficient of variation is effected by the optimum combination. The manner in which the coefficient of variation affects the accuracy of the estimation, as measured by the range within which the fruit content is likely to lie, has been previously shown.[1] For the sample of strawberry pulp considered above (p. 430), the limits within which the fruit content is likely to lie (probability = 0·95) are 64·7 and 98·5 per cent. Although the range is large the apparent fruit content of 78·1 per cent. is a more reliable estimate than the one obtained by averaging the two estimates obtained from the soluble solids and free acid separately. As remarked above, this estimate is equivalent to the use of a combination in which each constituent is divided by its mean value in the fruit. The coefficient of variation of such a combination may be found by inserting the value $\bar{x}_1/\bar{x}_2$ for k, giving $\frac{1}{2}\sqrt{V_1^2 + V_2^2 + 2rV_1V_2}$. Limits to the apparent fruit content of 80·5 per cent. are then 62·4 and 113·3 per cent. The estimate from the average is, in fact, less reliable than that from the use of the soluble solids figure alone, owing to too great emphasis being placed on the free acid content, which in strawberries has an extremely high coefficient of variation.

Although two constituents can, therefore, always be combined to form an optimum combination as shown, it does not follow that their combined use will necessarily lead to a more accurate estimation than by the use of one of them alone. In fact, whilst the coefficient of variation of the optimum combination can never be greater than that of either of the constituents, it may sometimes be equal to the lower one, and then there is obviously no advantage in carrying out both analytical determinations. With strawberries, for example, equal accuracy in estimating fruit contents is obtained by the use of soluble solids determination alone. Whether or not the optimum combination results in improved accuracy can always be seen by comparing the coefficients of variation. As mentioned above, the only case where no improvement occurs is where the ratio of the smaller to the larger coefficient of variation of the two constituents equals the correlation coefficient. In all other cases some improvement must occur, but this will not be appreciable (as with strawberries) unless these two values differ considerably. A negative correlation between the constituents always enables an improvement to be made. However, working out the optimum combination is worth while, even if no improved accuracy results, since it then establishes that one of the analytical determinations may be omitted without increasing the error of the estimation.

EXTENSION TO MORE THAN TWO CONSTITUENTS

Where other constituents are also available for determining the proportion of an ingredient, any pair can be treated as above, and the two constituents showing least variation in their optimum combination could then be selected for use. A more general line of approach is to find the optimum combination of all the available constituents taken together. This involves a more laborious calculation but can lead to a lower coefficient of variation than is obtainable from any pair of constituents.

As before, determinations of each constituent are required on the *same* samples of ingredient in order that their correlations may be calculated. The number of correlation coefficients necessary increases rapidly as more constituents are added. Thus four constituents involve six correlations, whilst five would require ten correlation coefficients. But it is rare for more than four constituents to be useful for estimating an ingredient. With fruit, data for the four constituents insoluble solids, soluble solids, sugars and free acid have been collected in these laboratories. The method of combining four measurements will be given; the treatment is general and can be extended to any number of constituents.

In generalising the procedure to more than two constituents the device of putting the first multiplying factor equal to unity has not been followed, as the advantage of symmetry in the resulting equations would be lost by so doing. The method employed is similar to the technique of multiple regression.[3] Calculation of the actual correlation coefficients may be avoided, though the corresponding cross-products are required.

Let x_1, x_2, x_3, x_4 denote the values of four different constituents (determined on the same sample of the ingredient). Also let $S_{11} = \Sigma(x_1 - \bar{x}_1)^2$, $S_{12} = \Sigma(x_1 - \bar{x}_1)(x_2 - \bar{x}_2)$, etc., where the summations extend over the n samples of the ingredient analysed, and $\bar{x}_1$, $\bar{x}_2$, etc., denote the means for the respective constituents. Then if the combination $x_c = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4$, where a_1, a_2, a_3 and a_4 are constants, the coefficient of variation of x_c is given by:

$$V_c = \frac{100s_c}{\bar{x}} = \frac{100\sqrt{(a_1^2S_{11} + \ldots + a_4^2S_{44} + 2a_1a_2S_{12} + \ldots + 2a_3a_4S_{34})/(n-4)}}{a_1\bar{x}_1 + a_2\bar{x}_2 + a_3\bar{x}_3 + a_4\bar{x}_4} \qquad (3)$$

The divisor (n — 4) is used in the numerator because four constants are now being fitted to the data, giving (n — 4) degrees of freedom for estimating the variance of the combination.

Values of a_1, a_2, etc., that make V_c a minimum are found by partial differentiation of the right-hand side of (3) with respect to each factor in turn and equating to zero, leading to the set of equations (apart from common factors):

$$\left.\begin{aligned} a_1S_{11} + a_2S_{12} + a_3S_{13} + a_4S_{14} &= \Sigma x_1 \\ a_1S_{12} + a_2S_{22} + a_3S_{23} + a_4S_{24} &= \Sigma x_2 \\ a_1S_{13} + a_2S_{23} + a_3S_{33} + a_4S_{34} &= \Sigma x_3 \\ a_1S_{14} + a_2S_{24} + a_3S_{34} + a_4S_{44} &= \Sigma x_4 \end{aligned}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

These equations may be solved in any of the standard ways of dealing with multiple regression equations. For reasons given below, it is best to use the c-matrix method of Fisher. Full working details of this method of solution are given in statistical works[3,4] and will be illustrated below with data from analyses of blackcurrants. Solutions to (4) can be written:

$$\left.\begin{aligned} a_1 &= c_{11}\Sigma x_1 + c_{12}\Sigma x_2 + c_{13}\Sigma x_3 + c_{14}\Sigma x_4 \\ a_2 &= c_{21}\Sigma x_1 + c_{22}\Sigma x_2 + c_{23}\Sigma x_3 + c_{24}\Sigma x_4 \\ a_3 &= c_{31}\Sigma x_1 + c_{32}\Sigma x_2 + c_{33}\Sigma x_3 + c_{34}\Sigma x_4 \\ a_4 &= c_{41}\Sigma x_1 + c_{42}\Sigma x_2 + c_{43}\Sigma x_3 + c_{44}\Sigma x_4 \end{aligned}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

Here the factors c_{11}, c_{12}, c_{13}, c_{14} are the values of a_1, a_2, a_3, a_4 that satisfy the four simultaneous equations obtained from (4) when Σx_1 is replaced by 1 and Σx_2, Σx_3 and Σx_4 by 0. The factors c_{21} to c_{24} are obtained by replacing Σx_2 by 1 and the others by 0 and solving the equations. Similar procedures give the values of c_{31} to c_{34} and c_{41} to c_{44}. Since it may be shown that $c_{12} = c_{21}$, $c_{13} = c_{31}$, etc., only ten c-factors need be calculated in theory. In practice, however, these equalities provide useful checks on the arithmetic.

If we multiply the first of equations (4) by a_1, the second by a_2 and so on, then adding up the left-hand side and dividing by the appropriate number of degrees of freedom gives the variance of the optimum combination. As four constants are fitted, (n − 4) degrees of freedom are available, hence:

$$(n - 4) \text{ var } x_c = a_1\Sigma x_1 + a_2\Sigma x_2 + a_3\Sigma x_3 + a_4\Sigma x_4$$

or

$$s_c = \sqrt{n\bar{x}_c/(n - 4)}$$

This gives a simple formula for calculating the coefficient of variation of the optimum combination as

$$V_c = 100s_c/\bar{x}_c = 100/\sqrt{\frac{n-4}{n}(a_1\bar{x}_1 + a_2\bar{x}_2 + a_3\bar{x}_3 + a_4\bar{x}_4)} \quad .. \quad .. \quad (6)$$

There is a resemblance between the combination of measurements to give minimum coefficient of variation and the discriminant function introduced by Fisher. The latter quantity represents the combination of various measurements that best discriminates between two different varieties, or species. For example, we might be interested in the combination of insoluble solids and soluble solids that would best distinguish blackcurrants from strawberries (if no other means of identification were available). The present combination may be regarded as the discriminant function that best distinguishes the original fruit from the population of all pulps containing a fixed proportion, P, of fruit. The coefficients calculated for this discriminant are found to be proportional to the coefficients as calculated above (for any given P). Since any set of constants *proportional* to a_1, a_2, etc., leaves the coefficient of variation of the combination unchanged, the discriminant function interpreted in this way leads to the same solution for the best linear combination as above.

The values of the coefficients calculated from equations (5) may be in error from the true values which would be obtained if all possible samples of the ingredient could be analysed. In practice, only a small amount of data may be available, particularly as only data from samples in which all constituents have been determined can be utilised. In many cases the correlations are not statistically significant although their numerical values are incorporated in the calculation. From analogy with the discriminant function, the standard error of the coefficients a_1, a_2, etc., may be easily calculated by multiplying the standard error s_c of the combination by $\sqrt{c_{11}}$, $\sqrt{c_{22}}$, etc.

Equations (5) and (6) above enable the multiplying factors and the coefficient of variation of the optimum combination to be worked out for the four constituents. When the optimum combination is evaluated it is necessary to decide whether all the measurements involved are useful in lessening the variation. This is not obvious, as it is with two constituents only, because even if the coefficient of variation of the combination is less than that of any one constituent this may be due entirely to the combined effect of only three or perhaps two constituents. Any constituents that are not useful can be omitted in the chemical analysis without reducing the accuracy of the final estimate of the percentage of ingredient. A criterion for rejecting or retaining any constituents in the combination is provided, however, by the standard errors of their coefficients a_1, a_2, etc. Any constituent whose coefficient is numerically less than its standard error can be omitted without increasing by more than 1 per cent. the coefficient of variation of the optimum combination of the remaining constituents. These standard errors can be easily calculated, as indicated above, if Fisher's c-matrix method of solving equations (4) is employed.

If more than 50 observations are involved, the coefficient needs to be approximately $\sqrt{n}/7$ times its standard error for the constituent to be usefully retained in the combination. The exact value for the estimated coefficient of variation of the optimum combination after rejecting any constituent can be calculated from the value before rejection by dividing by the factor

$$\sqrt{\frac{n-p+1}{n-p}\left\{1 - \frac{a^2}{(n-p)\text{ var } a}\right\}}$$

where a = value of the coefficient of the rejected constituent, n = number of observations and p = number of constituents combined (before rejection).

When a constituent is discarded it becomes necessary to recalculate the coefficients for the optimum combination of the remaining constituents. There is no need, however, to solve the original equations afresh, leaving out the unwanted measurement. If any constituent, say x_p, is to be rejected, then the new c-matrix may be calculated[3] from the old by relations of the type $c'_{ij} = c_{ij} - \frac{c_{ip}\, c_{jp}}{c_{pp}}$, where c'_{ij} is the new value. The appropriate values of the new coefficients, with their standard errors, then follow as above, except that one more degree of freedom is now available.

It is the advantage of the c-matrix method of solution that it enables the standard errors of the coefficients to be readily calculated and also permits easy adjustment of their values when one or more constituent is discarded for any reason. In practice it is possible to round off the calculated values of the coefficients or to reduce them, in approximate proportion, to small whole numbers without appreciably altering the coefficient of variation of the combination. It is desirable for the coefficients to be small integers, if possible, in order to minimise the arithmetical labour of calculating the proportion of ingredient. The final coefficient of variation of the combination adjusted in this way must, however, be checked from the expression (3) and not from equation (6), inserting the modified coefficients. This should not differ from the optimum value by more than, say, 1 per cent.

APPLICATION TO BLACKCURRANTS AND OTHER FRUITS—

The following data from 20 samples of blackcurrants illustrate the method of arriving at the optimum combination of the constituents insoluble solids, soluble solids, sugar and free acid. Table II shows the sum of squares and cross-products (of deviations from the means) for the four constituents, which are the values S_{11}, S_{12}, etc., above. Thus the entry of 66·116 in column 3, line 2 (or column 2, line 3) denotes the cross-product $S_{23} = \Sigma(x_2 - \bar{x}_2)(x_3 - \bar{x}_3)$ of soluble solids and sugars. The table is symmetrical about the diagonal, since $S_{ij} = S_{ji}$, where S_{ij} is the value in column i, row j. The last row in Table II shows the sums Σx of each constituent for the 20 samples.

TABLE II

SUMS OF SQUARES AND CROSS-PRODUCTS OF FOUR CONSTITUENTS (MEASURED FROM MEANS) IN 20 SAMPLES OF BLACKCURRANTS

	Insoluble solids x_1	Soluble solids x_2	Sugars x_3	Free acid x_4
x_1	+ 11·119	− 7·035	− 7·207	+ 1·360
x_2	− 7·035	+ 71·214	+ 66·116	− 1·721
x_3	− 7·207	− 66·116	+ 81·099	− 8·573
x_4	+ 1·360	− 1·721	− 8·573	+ 2·890
Σx	117·4	290·4	132·0	70·6

Values for the c-matrix are then calculated by solving the four sets of four equations, equating the left-hand side to each column of the right-hand side in turn.

$$\left.\begin{array}{r} 11{\cdot}119\,a_1 - 7{\cdot}035\,a_2 - 7{\cdot}207\,a_3 + 1{\cdot}360\,a_4 = 1,\ 0,\ 0,\ 0 \\ -7{\cdot}035\,a_1 + 71{\cdot}214\,a_2 + 66{\cdot}116\,a_3 - 1{\cdot}721\,a_4 = 0,\ 1,\ 0,\ 0 \\ -7{\cdot}207\,a_1 + 66{\cdot}116\,a_2 + 81{\cdot}099\,a_3 - 8{\cdot}573\,a_4 = 0,\ 0,\ 1,\ 0 \\ 1{\cdot}360\,a_1 - 1{\cdot}721\,a_2 - 8{\cdot}573\,a_3 + 2{\cdot}890\,a_4 = 0,\ 0,\ 0,\ 1 \end{array}\right\} \quad \ldots \quad \ldots \quad (7)$$

These values are shown in Table III (mathematically the inverse matrix of Table II). Again the table is diagonally symmetrical, as $c_{ij} = c_{ji}$.

TABLE III

c-FACTORS CALCULATED FROM EQUATIONS (7)

	x_1	x_2	x_3	x_4
x_1	+ 0·12447	+ 0·10260	− 0·10535	− 0·30988
x_2	+ 0·10260	+ 0·38463	− 0·41567	− 1·05192
x_3	− 0·10535	− 0·41567	− 0·46749	+ 1·18844
x_4	− 0·30988	− 1·05192	+ 1·18844	+ 3·38983

Equations (5), in which c_{ij} is the figure in row i, column j, of Table III, may now be solved, giving for the coefficients $a_1 = 8\cdot624$, $a_2 = -5\cdot392$, $a_3 = 12\cdot534$ and $a_4 = 54\cdot339$. From these we calculate $n\bar{x}_c = 1012\cdot458 - 1565\cdot837 + 1654\cdot488 + 3836\cdot333 = 4937\cdot442$, whence $\bar{x}_c = 246\cdot872$, $s_c = \sqrt{4937\cdot442/16} = 17\cdot57$ and $V_c = 7\cdot12$.

The standard errors of the coefficients are 6·20 ($= 17\cdot57 \times \sqrt{0\cdot12447}$), 10·89, 12·01 and 32·34 respectively. The standard error of a_2 is about twice its numerical value, so that the soluble solids constituent may be omitted from the combination. A recalculation of the c-matrix, omitting x_2, is therefore made (see p. 435) by means of equations of the type $c'_{ij} = c_{ij} - (c_{i2}c_{j2}/0\cdot38463)$.

These values are shown in Table IV.

TABLE IV

c′-FACTORS CALCULATED FROM TABLE III WHEN x_2 IS OMITTED

	x_1	x_3	x_4
x_1	+ 0·09710	+ 0·00553	− 0·02928
x_3	+ 0·00553	+ 0·01827	+ 0·05163
x_4	− 0·02928	+ 0·05163	+ 0·51294

Values of the coefficients with their standard errors are now $a_1 = 10\cdot062 \pm 5\cdot27$, $a_3 = 6\cdot706 \pm 2\cdot28$, $a_4 = 39\cdot591 \pm 12\cdot11$, and the coefficient of variation of the combination is 6·96. This is actually less than the optimum value with all four constituents, owing to the fact that the variance is now estimated from 17 instead of 16 degrees of freedom. All coefficients are now greater than their standard errors and the three constituents must all be regarded as useful in reducing the variation of the combination. The coefficient a_1 of the insoluble solids has the greatest proportional standard error, but it cannot be omitted from the combination without increasing the estimated variation (coefficient of variation of the optimum combination of sugars and free acid alone = 7·63). Thus, on the evidence available from the analysis of 20 samples of blackcurrants, the maximum precision in analysis arises from the use of the combination [10·1 (insoluble solids) + 6·7 (sugars) + 39·6 (free acid)] and no further improvement is achieved by including a determination of soluble solids.

If all four analytical determinations are made on a blackcurrant pulp and an average is taken of the estimates of fruit content given by each constituent separately, it may be shown that this is equivalent to using a single combined factor with a coefficient of variation of 9·81. Thus the above statistical treatment enables a more accurate estimation of the fruit content to be made with one less analytical determination, and makes more efficient use of the available data.

It may be that when a constituent is rejected a larger number of samples becomes available for calculating the optimum combination of the remaining constituents. In this event a recalculation should be made, and the new values employed, even though the additional data happen to lead to an increase in the estimated coefficient of variation of the combination. Such an increase (or decrease) is quite likely to occur through sampling variations. In calculating the mean value of the optimum combination, the mean value inserted for each constituent should be estimated from as large a number of samples as possible. For this purpose it is not necessary to restrict the data to samples on which all constituents have been determined. Such a mean value, of course, cannot be used in calculating the coefficient of variation of the optimum combination, the best estimate of which is already obtained as above. But *solely* for the purpose of calculating the percentage of ingredient this will be the most accurate mean value to use.

Table V has been drawn up in this way, and gives for various fruits the optimum combination out of the four determinations insoluble solids, soluble solids (by specific gravity), sugars (as invert sugar) and free acid (as hydrated citric acid) together with the mean value of the combination based on data collected over a number of years. The reducing sugars were determined by Fehling's titration on a suitably diluted aqueous extract of the fruit. The other analytical determinations were carried out as described by Hinton and Macara.[2] The exact values of the factors have been rounded off, as explained above, to give integral values as small as possible without significantly increasing the coefficient of variation of the combination. It should be noted that some of the combinations involve the difference between constituents rather than their sum. This is the case where a multiplying factor turns out to be negative owing to a relatively large positive correlation between the constituents. The numerical value of the whole combination is, of course, always positive.

The table also shows the coefficient of variation, V_c, of the optimum combination, together with the number of samples, n, on which it is based. For comparison are shown the coefficient of variation, V_s, of the best single constituent and the coefficient of variation, V_a, of the combination equivalent to averaging estimates from all four single constituents.

TABLE V

OPTIMUM COMBINATION OF THE CONSTITUENTS INSOLUBLE SOLIDS, SOLUBLE SOLIDS, SUGARS AND FREE ACID (AS HYDRATED CITRIC ACID)

	Optimum combination				Best single factor		
Fruit	Factors	Mean value	V_c	n	Constituent	V_s	V_a
Apple	Sol. solids + 2 (free acid)	14·2	7·4	37	Sol. solids	8·4	11·2
Blackcurrant	Insol. solids + sugar + 6 (free acid)	32·7	7·0	20	Free acid	11·0	9·8
Damsons	3 (Sol. solids) — 4 (sugar) + 9 (free acid)	38·8	9·6	10	Free acid	15·0	14·7
Gooseberry	Insol. solids + 6 (soluble solids) — 3 (sugar)	42·4	7·2	48	Sol. solids	9·7	12·2
Plum	9 (Insol. solids) + 2 (sol. solids) + 3 (free acid)	41·7	15·2	41	„	17·9	15·2
„ (sieved)	Sol. solids + 2 (free acid)	16·1	17·2	50	„	17·9	—
Raspberry	5 (Sol. solids) — 4 (sugar) + 4 (free acid)	33·5	11·8	29	„	17·4	16·3
Strawberry	Insol. solids + sol. solids	11·2	9·8	65	„	10·5	11·1

The optimum combination for sieved plums has been included in view of the common practice of sieving this fruit. This has been calculated by omitting the insoluble solids factor and recalculating the combination for the remaining three constituents in the manner indicated above. Strictly speaking, therefore, this does not represent the optimum combination of the four constituents, but is the best available in the absence of data for insoluble solids in sieved plums.

CONCLUSIONS

From a consideration of Table V it is evident that for the common fruits not more than three (in some cases only two) constituents need be determined in a pulp to obtain the percentage of fruit with the maximum precision. The gain in precision (as measured by the coefficient of variation) over the use of the best single constituent varies from about 36 per cent. for blackcurrants and damsons to only 4 per cent. for sieved plums. It is interesting to note that out of the four constituents, the soluble solids appears as the most reliable by itself in all cases except two. It should be noted that if separate estimates from all four constituents are averaged, the percentage of fruit may be obtained with *less* precision (indicated by V_a) than if one constituent only is used (V_s). This is notably so with apples and gooseberries, owing to the relatively large variation in some constituents.

In order to obtain the most reliable estimate, therefore, of the fruit content of a pulp the constituents shown in the combinations of Table V are determined in the pulp, multiplied by the factors given, and divided by the mean value shown. Limits to the estimate may be calculated from the coefficient of variation V_c, bearing in mind that this is based on (n — 4) degrees of freedom.

Consideration of the optimum combination has been confined to the examples of fruit, but it is of perfectly general application. The combination represents that linear function of the constituents which is most constant in samples of the ingredient. This provides the most reliable factor for estimating the percentage of ingredient in a food product if each constituent, taken by itself, leads to an estimate of the percentage in direct proportion to the amount present. It is assumed also that the amount of constituent in the food product is known exactly, that is, that no analytical errors or other uncertainties affect its determination. If this does not hold, the combination worked out to show minimum variation in samples of the ingredient may not also show minimum variation in samples of the food product containing a certain fixed proportion of ingredient, and consequently may not lead to the most reliable estimate. Considerations of this type arise with jams where uncertainty enters into the analytical determinations largely owing to use of added pectin. Sampling errors may also be appreciable, particularly in the insoluble solids determination, owing to non-uniform filling into jars from a batch of jam. For these reasons the case of jam is more complex and requires separate treatment, which it has not yet been possible to give owing to lack of suitable data.

Apart from these limitations, the optimum combination can always be used where two or more constituents, each of which show variation in the ingredient, are available for the determination of the ingredient. For more than two constituents the calculation becomes somewhat tedious unless a machine is available, but the gain in precision may make the time spent well worth while, particularly if some analytical determinations may subsequently be omitted as a result.

SUMMARY

A linear combination of two or more chemical constituents of an ingredient may be calculated that shows less variation in samples of the ingredient than any other linear combination. If the value of this optimum combination is determined for a sample of a food product and divided by the mean value for the ingredient the most reliable estimate of the true proportion of ingredient is obtained. Where several constituents are available for the purposes of the estimation it is possible to reject any that do not contribute to the reliability of the estimate. The use of the optimum combination may enable greater accuracy to be achieved, with fewer analytical determinations, than if estimates from all available constituents are averaged. The method is applicable to all food products where the whole of the values of the chemical constituents determined are contributed by the ingredient estimated. The calculation of the optimum combination is illustrated for blackcurrants and the combination given for various fruits, applicable to the analysis of pulps.

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A Study of the Methods Prescribed by the F. & F. S. Regulations (1932) for the Estimation of Oil, Albuminoids and Fibre in Feeding Stuffs

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DAILY use, over many years, of the methods for estimating oil, albuminoids and fibre in feeding stuffs, laid down in the Fertilisers and Feeding Stuffs Regulations, 1932 (S.R. & O., 1932, No. 658), has shown the urgent need for their reconsideration. These methods are dealt with in turn below.

ESTIMATION OF OIL

The Regulations state—

(*a*) "A weighed quantity of the sample shall be placed in an extraction thimble, which shall then be placed in an extraction apparatus and extracted with petroleum spirit, b.p. 40° to 60° C. At the end of 3 to 4 hours the thimble shall be removed from the apparatus, dried and its contents finely ground, preferably with sand, in a small mortar previously rinsed with petroleum spirit. The substance shall then be returned to the thimble, the mortar being washed out with petroleum spirit, and the extraction containued for another hour. The extract should be free from suspended matter. After evaporation of the solvent, the oil shall be dried at 100° C. and weighed.

(*b*) In the case of samples containing saccharine matter, the weighed portion in the thimble shall be washed with water and then dried, previous to extraction."

It is assumed that procedure (*b*) must be applied to all compound feeding stuffs containing molasses and procedure (*a*) to all other feeding stuffs. It might, however, be contended that practically all feeding stuffs should be water-washed, for most of them contain natural sugars, as shown in Table I.

TABLE I

NATURAL SUGARS IN FEEDING STUFFS

Material	Sugars as cane sugar %	Material	Sugars as cane sugar %
Barley	1 to 2	Milk whey dried	40 to 45
Bran (wheat)	5 to 6·5	Oats	1 to 3
Beet residue	3 to 9	Palm kernel cake	2 to 3
Coconut cake (copra)	10 to 15	Potato flakes	3 to 4
Carrot pomace	24	Rape meal	4 to 6
Cocoa cake	4	Rice	0 to 1
Cotton cake (undecorticated)	3 to 5	Rice bran	2 to 3
Fish meal	nil	Rice bran (parboiled)	0 to 1
Groundnut cake (decorticated)	6 to 9	Soya bean meal	9 to 11
Linseed cake	5 to 6	Sunflower cake	5 to 6
Locust bean meal	38 to 42	Tapioca	0 to 3
Maize meal	1 to 3	Wheat	2 to 3
Maize gluten	0 to 1	Wheat flour (seconds)	3 to 5
Milk powder (skimmed)	30 to 35		

These figures for sugar content show the ranges found in the course of our experience and they are in broad agreement with those in the scientific literature.

Owing to the presence of sugars in most feeding stuffs it appeared desirable to ascertain (1) how different types of feeding stuffs behave when subjected to the water-washing process and (2) what is the percentage of molasses above which it is necessary to water-wash in order to obtain the correct figures for oil. With that object, estimations of oil were made on the following feeding stuffs, with and without previous water-washing.

	Natural sugars %	Added molasses %	Total sugars %	Added calcium carbonate %
Palm kernel meal	3·2	nil	3·2	1
Mixed bran and palm kernel meal (1 + 1)	4·7	nil	4·7	0·5
Poultry meal	6·0	nil	6·0	2·8
Poultry pellets	5·3	3·0	6·8	2·8
National Cattle Food*	4·3	5·0	6·8	2·0

* Contained 17·5 per cent. of linseed cake.

In the oil estimations the thimble specified in the Regulations was not used, but the meal was wrapped in filter paper and placed in a Tate tube for extraction.

Water-washing of the samples was carried out as follows: 5 g. of the meal were stirred in a beaker with 5 ml. of cold water, filtered and washed twice, each time with 25 ml. of cold water. The damp meal was transferred to a basin and dried on a steam-bath, the filter paper being dried in a steam-oven. The drying time was in this way reduced to 1 hour and the difficulty due to the meal adhering strongly to the paper was avoided. After drying, the meal was transferred to the dry filter paper, wrapped in another filter paper and extracted. The residue was re-ground and re-extracted.

The results are shown in Table II.

The observed standard errors were calculated: for direct extraction, A, the average standard error was ±0·044 per cent. and that for extraction after water-washing, B, ±0·059

Table II

Yields of oil, per cent.

A. From sample direct. B. From water-washed sample

	Palm kernel meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal No. 1	
	A	B	A	B	A	B	A	B	A	B
Replicate determinations	9·74	9·35	7·58	6·92	6·34	5·46	5·52	4·80	6·36	5·66
	9·74	9·45	7·66	7·00	6·32	5·48	5·48	4·73	6·40	5·64
	9·79	9·52	7·56	6·85	6·26	5·52	5·54	4·82	6·29	5·64
	9·80	9·46	7·58	6·81	6·32	5·44	5·54	4·83	6·34	5·66
			7·56	6·80					6·36	5·64
			7·66						6·41	5·62
Means	9·77	9·45	7·60	6·88	6·31	5·48	5·52	4·80	6·37	5·64
Difference A − B	0·32		0·72		0·83		0·72		0·73	

per cent. Direct extraction gave more consistent results than extraction after water-washing, but both methods were satisfactory in respect of standard error. Analysis of variance showed that variations between the methods far outweighed the variations caused by the experimental errors of each method.

These results have been confirmed in a number of laboratories in our organisation. Table III shows the extent to which the water-wash method resulted in low oil figures for 78 samples of feeding stuffs of various types containing low percentages of added molasses.

Table III

Oil per cent. found by direct extraction minus oil per cent. found after water-washing	Number of samples
0·25 to 0·50	17
0·51 to 0·75	23
0·76 to 1·00	20
1·01 to 1·50	13
above 1·50	5

It has thus been established that feeding stuffs with or without added molasses can yield appreciably lower oil figures after the water-wash treatment, prescribed by the Feeding Stuffs Regulations.

Experiments were made to ascertain the cause of the difference, attention being directed to possible changes in free fatty acid content, loss of oil by emulsification, formation of lime soap and the effect of moisture.

FREE FATTY ACID CONTENT OF THE EXTRACTED OILS—

The free fatty acid contents of the oils extracted directly or after the water-wash were determined, the true molecular weights being calculated from the saponification equivalents. The figures, which refer to the same feeding stuffs as were used in the preceding experiments, are set out in Table IV and related to the loss of oil in the water-washing process.

Table IV

Free fatty acids of extracted oils

A. From sample direct. B. From water-washed sample

	P.K. meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal	
	A	B	A	B	A	B	A	B	A	B
F.F.A. in extracted oil, %	6·1	4·6	12·5	7·9	15·4	10·0	16·8	13·8	18·1	13·7
F.F.A., calculated on meal, %	0·60	0·43	0·88	0·54	0·97	0·55	0·98	0·66	1·15	0·77
Deficiency of oil due to water-washing, % on meal	0·32		0·72		0·83		0·72		0·72	
Deficiency of F.F.A. due to water-washing, % on meal	0·17		0·34		0·42		0·32		0·38	

The oil extracted after washing contained appreciably less free fatty acid than the oil obtained by direct extraction and the extent of the reduction of the free fatty acid content corresponded to about half of the oil lost by the water-washing process. No appreciable amount of fatty matter could be extracted from the filtrate from the water-wash.

POSSIBLE FORMATION OF CALCIUM SOAP DURING WATER-WASHING—

Addition of calcium carbonate to certain stock foods had been customary in the industry for a number of years, and in 1940 its addition to certain feeding stuffs became compulsory. In July, 1942, this regulation became general, apart from special foods such as calf meals and foods for which a special licence has been obtained. Hence there arises the possibility of a reaction between the calcium carbonate in the cattle food and free fatty acid in the oil when the meal is in the wetted condition in the water-wash process. The residues after direct extraction and after water-washing and extraction were moistened with water followed by a small quantity of concentrated hydrochloric acid, and then dried on the water-bath and re-extracted. Under these conditions the figures shown in Table V were obtained.

TABLE V

EFFECT OF ACIDIFICATION AND RE-EXTRACTION

A. Sample direct. B. Water-washed sample

	P.K. meal		Bran and P.K. meal		Poultry meal		Poultry pellets		N.C.F. meal	
Treatment	A	B	A	B	A	B	A	B	A	B
Straight extraction ..	9·73	9·39	7·60	6·88	6·19	5·21	5·30	4·68	6·22	5·45
Further grind plus 1-hour extraction	0·04	0·06	0·05	0·04	0·12	0·27	0·22	0·12	0·15	0·19
Acidification and re-extraction	0·08	0·19	0·41	0·67	0·34	0·57	0·11	0·42	0·11	0·26

After acidification the residue from the first extraction of the water-washed meal yielded more fatty matter than that from the directly extracted meal. This additional fatty matter was found to contain appreciable quantities of fatty acid. These effects may be connected with the formation of lime soaps from the calcium carbonate and the free fatty acid in the wetted mass.

EFFECT OF DRYING ON OIL EXTRACTION—

Oil extractions were carried out on various meals, with and without prior drying, Series I for 3 hours at 102° C. and Series II for 2 hours at 105° C. The results are shown in Table VI.

TABLE VI

EFFECT OF DRYING ON OIL EXTRACTION

	Moisture	Oil: on meal as received	Oil: on dried meal calculated to original meal
		%	%
Series I			
Palm kernel meal	10·3	9·77	9·74
Bran plus P.K. meal	11·0	7·6	7·38
Poultry meal	10·9	6·31	6·14
Poultry pellets	11·8	5·52	5·46
N.C.F. meal	12·3	6·36	6·13
Series II			
Pig Meal No. 1	10·8	3·95	3·6
Calf Nutlettes	11·75	6·4	6·45
Poultry Mash No. 1	10·9	3·0	2·8
National Cattle Nuts No. 1A ..	12·5	3·9	3·5
Linseed cake..	10·8	9·55	9·3
Extracted palm kernel meal ..	8·4	1·5	1·55
Extracted rape meal	9·8	2·2	2·2
Fish meal	7·75	6·85	5·65
Bran	10·7	4·4	4·25
Decorticated groundnut cake ..	8·75	9·5	9·5

The figures show that considerable reduction in oil content can result from drying the meal, which is a necessary part of the water-wash process.

A serious objection to the water-wash process is that some of the feeding stuffs, *e.g.*, those containing linseed cake, are apt to form gelatinous masses that are difficult to handle and to filter.

POSSIBLE LOSS THROUGH CHANGES IN THE DAMP MEAL—

In some cases during the water-wash process the meal may remain in a wet condition for a comparatively long time. This may result in an increase in free fatty acid and other changes, including the formation of lime soap. In order to get some idea of the effects of leaving the meal in a wet condition, washed poultry meal was spread on a dish and allowed to dry in an evacuated desiccator; with fresh sulphuric acid the drying took 48 hours and with once-used sulphuric acid 4 days. The effect is shown in Table VII.

TABLE VII

Treatment of sample	Period during which sample was damp	Oil, %
Extracted directly	—	6·3
Washed, dried at 102° C. . .	3 hours	5·47
Washed, dried in desiccator . .	48 hours	5·12
Washed, dried in desiccator . .	4 days	3·58
Washed, and later dried at 102° C.	4 days	2·54

In keeping the washed meal damp for 4 days, the storage conditions were purposely exaggerated, but the figures show clearly that in the wet condition changes do occur that lead to serious reduction in the figures for oil content.

The preceding sections have shown that the low oil figures obtained after water-washing can be partly due to (1) the formation of calcium soaps in the wet mass, (2) the effect of drying the wet mass before extraction with solvent and (3) the effect of changes in the wet mass if it is allowed to remain unduly long in the wet condition.

EFFECT OF VARIOUS PROPORTIONS OF MOLASSES UPON OIL EXTRACTION—

To determine the effect of different proportions of molasses upon the apparent oil content, palm kernel cake meal (without added calcium carbonate) and mixtures of the meal with molasses were extracted with light petroleum (b.p. 40° to 60° C.) directly and after water-washing. The oil content found by direct extraction of the meal without molasses was taken as the "theoretical" value and used as the basis for calculating the theoretical oil contents of the molassed samples, given in the second column of Table VIII (upper part).

TABLE VIII

Extraction with light petroleum (b.p. 40° to 60° C.) only
Oil content of original meal by direct extraction 7·05%

				Theoretical figure minus	
Molasses in sample %	Oil in sample, theoretical %	Oil by direct extraction %	Oil by extraction after water-wash %	Direct figure %	Water-wash figure %
0	—	7·05	6·55	—	0·50
15	5·99	5·83	5·58	0·16	0·41
20	5·64	5·43	5·44	0·21	0·20
25	5·29	4·96	5·07	0·33	0·22
30	4·93	4·47	4·82	0·46	0·12

Extraction with methylated ether followed by extraction of resulting oil with light petroleum
Result on original meal 7·28% oil

Molasses in sample %	Oil in sample, theoretical %	Oil found %	Theoretical figure minus figure found %
0	—	7·28	—
15	6·18	6·03	0·15
20	5·82	5·90	— 0·08
25	5·46	5·60	— 0·14
30	5·10	5·18	— 0·08

A similar set of estimations was carried out on the mixtures after they had been cooked and shaken in their glass-stoppered bottles in a steam-oven for several hours, but these results are not quoted, as they were similar to those obtained on the uncooked mixtures.

The 1906 Act prescribed the use of methylated ether as a solvent and it was decided to carry out estimations by extraction with methylated ether in the first instance and after removal of the ether from the extract, extracting the latter with light petroleum (b.p. 40° to 60° C.); the idea was that the methylated ether would probably extract all the oil, plus a little sugar, and then the light petroleum would take up only the oil. It was realised that methylated ether by itself tends to give high figures for oil, sometimes 1 per cent. high.[1] The results are given in the lower part of Table VIII.

From the first set of figures, with light petroleum alone, it will be seen that direct extraction gives a slightly low oil figure at 20 per cent. of molasses, but decidedly low figures at 25 and 30 per cent. of molasses, whilst the water-wash method gives very low figures at 0 and 15 per cent. of molasses and a reasonably accurate figure at 30 per cent. Hence highly molassed feeds should be water-washed before extraction. The dividing line, above which water-washing should be practised, appears to be about 25 per cent. of molasses. With the second method reasonably accurate results are obtained at all the levels of molasses tested. Similar results were obtained on mixtures of palm kernel cake and molasses, to which calcium carbonate had been added.

SUMMARY—

The water-washing method is cumbersome and lengthy and its effect, whether or not the feeding stuffs contain added molasses, is to reduce the apparent oil content to a varying and unpredictable degree, which may be as much as 1·5 per cent. This reduction is associated with formation of soap from the calcium carbonate and the free fatty acid in the oil, lowered extraction efficiency on dried meal and possible changes in the wet mass on standing.

When the proportion of molasses does not exceed 25 per cent., direct extraction is satisfactory; with higher percentages it is desirable to wash out the molasses before extracting the oil, or alternatively, the oil can be first extracted with methylated ether, the solvent evaporated from the extract and the latter extracted with light petroleum. The use of two solvents would be an added complication in carrying out numerous daily routine estimations.

ESTIMATION OF ALBUMINOIDS

The Fertilisers and Feeding Stuffs Regulations, 1932, prescribe the use of 25 ml. of concentrated sulphuric acid, 10 g. of potassium or sodium sulphate and a small crystal of copper sulphate or a globule of mercury in the Kjeldahl digestion flask. Mercury is well known as an effective catalyst for the digestion, but it requires addition of sodium sulphide to the liquor before distillation. In recent years, selenium has been coming into regular use in the Kjeldahl process and has been the subject of numerous publications.[2] Alcock gives figures to show the increasing rapidity of the process with increasing quantities of sodium sulphate in presence of 0·2 g. of copper sulphate.[3]

It was considered desirable to obtain further information on the effect of increasing quantities of selenium in presence of various quantities of sodium sulphate.

Kjeldahl determinations were carried out in the normal manner on 1 g. of high-protein cattle meal or 1 g. of dried pure sulphanilic acid, with either 8 or 18 g. of anhydrous sodium sulphate, 25 ml. of concentrated sulphuric acid and different quantities of selenium metal. For details of the test conditions see Table IX.

The figures for the protein meal in Table IX show the marked accelerating effect of selenium on the destruction of organic matter. Increasing the amount of sodium sulphate from 8 to 18 g. reduced the time of clearing in absence of catalyst and in presence of very small quantities of it (*e.g.*, 0·01 g. of selenium). Nothing appears to be gained by using more than 0·1 g. of selenium and there is some evidence that 0·2 g. gives slightly lower results.* Analysis of variance by Fisher's method indicated that in both sets of experiments significant increase in albuminoids found occurred when 0·1 g. of selenium was used (compared with 0·01 g.), and in the experiments with the protein meal when 3 hours boiling was given after clearing, instead of the 1 hour prescribed. Increase in the amount of sodium sulphate from 8 to 18 g. had no significant effect.

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* Patel and Sreenivasan (*Anal. Chem.*, 1948, **20**, 63) state that prolonged boiling with selenium leads to loss of nitrogen and that this can be corrected by the addition of mercuric oxide; their basal salt mixture contained copper sulphate, and thus they never worked with selenium as sole catalyst.

TABLE IX

DETERMINATIONS ON HIGH-PROTEIN MEAL AND ON SULPHANILIC ACID

1 g. of sample with 25 ml. of concentrated H_2SO_4 in Kjeldahl flask, with additions as below

Catalyst	Na_2SO_4 g.	Clearing time, min.	Additional heating time, min.	Total heating time, min.	Albuminoids %
Determinations on a high-protein meal					
None	8	120	60	180	35·29
None	18	95	60	155	35·36
Selenium metal, 0·01 g.	8	43	60	103	35·40
,,	8	40	180	220	36·09
,,	18	33	60	93	35·57
,,	18	36	180	216	36·10
0·05 g.	8	30	60	90	36·09
0·10 g.	8	25	60	85	36·09
,,	8	25	180	205	36·22
,,	18	25	60	85	36·02
,,	18	25	180	205	36·21
0·15 g.	8	25	60	85	35·84
0·20 g.	8	25	60	85	35·96
Copper sulphate, 0·1 g.	8	60	60	120	35·86
,,	8	60	180	240	36·00
,,	18	50	60	110	35·92
,,	18	50	180	230	36·14
Determinations on sulphanilic acid (N = 8·093%)					Nitrogen %
None	8	150	60	210	7·74
Selenium metal, 0·01 g.	8	30	60	90	7·88
,,	8	30	180	210	8·03
,,	18	30	60	90	8·04
,,	18	30	180	210	8·07
0·05 g.	8	30	60	90	8·00
0·10 g.	8	30	60	90	8·07
,,	8	30	180	210	8·07
,,	18	25	60	85	8·08
,,	18	25	180	205	8·06
0·15 g.	8	25	60	85	8·02
0·20 g.	8	25	60	85	8·01

Some further tests were carried out on blood meal and fish meal, which have high albuminoid contents and might conceivably present difficulties in obtaining accurate figures. Mercury (one globule) was included in these tests and copper sulphate was used at the augmented amount of 0·5 g. The results are shown in Table X.

TABLE X

ESTIMATION OF ALBUMINOIDS IN BLOOD MEAL AND FISH MEAL

	Found, per cent.		
Total time of heating	With 0·5 g. of mercury	With 0·5 g. of copper sulphate	With 0·1 g. of selenium
Blood meal:			
1½ hours	82·1	80·7	81·5
2½ ,, ...	82·2	81·5	81·5
3½ ,, ...	82·1	81·5	81·9
6 ,, ...	82·0	81·9	82·0
Fish meal:			
1½ hours	74·1	72·6	73·3
2½ ,, ...	73·8	73·3	74·0
3½ ,, ...	74·1	73·6	73·8
6 ,, ...	74·5	74·2	74·0

For blood meal the clearing times were much the same with each catalyst, about 25 minutes; but for the fish meal the mercury and the selenium caused clearing in about 25 minutes and the copper sulphate in 35 minutes.

It would appear that 0·5 g. of mercury is the best catalyst in the sense of yielding the maximum albuminoid figure in the shortest time and that, as in the previous tests, 0·1 g. of selenium is a distinctly better catalyst than copper sulphate. The Regulations specify that heating should be continued for 1 hour after clearing. Our results have shown that 0·1 g. of selenium requires some 2½ to 3 hours heating after clearing in order to obtain the maximum figure and that copper sulphate requires more time than selenium. Alcock[3] considers that it is important to specify a total digestion time rather than 1 hour after clearing; this opinion is supported by our figures for selenium and copper sulphate.

ESTIMATION OF FIBRE

For the estimation of fibre the Regulations provide that if the sample is sufficiently fine to pass through a sieve having apertures about 1 mm. square it should be used as it is; if it is not it should be powdered until it passes through the sieve.

In the first case the analyst must work with the material as received, but in the other the sample upon which the test is to be made may in the main be ground very finely or only just sufficiently so to pass through the sieve. Fineness of grinding is an important factor in the estimation of fibre and in the following tests the samples were sieved after each grinding, only the portion that failed to pass the sieve being reground. Except for the sample that was very finely ground (Table XIII), the sieve used was B.S.S. No. 16.

The method is empirical and therefore the results obtained are particularly dependent upon individual interpretation, in the absence of precise directions on details.

A few points call for special mention: (i) 2·5 or 3 g. of sample are taken; (ii) after removal of the oil the residue is dried (method not stated); (iii) during the treatment with acid and caustic soda, the contents of the flask are boiled gently and continuously for exactly 30 minutes; (iv) during these boilings the flask is to be rotated every few minutes in order to mix the contents and remove particles from the sides.

A series of co-operative tests was undertaken in nine separate laboratories where feeding stuffs were daily analysed. The results are summarised in Table XI.

TABLE XI

Feeding stuff	Number of labs.	Number of operators	Number of tests	Fibre, %			Highest minus Lowest result
				Highest	Lowest	Average	
Bran A	8	—	39	10·7	9·0	10·0	1·7
Bran B	9	21	42	12·12	10·03	10·92	2·09
Extracted palm kernel meal:							
Sample A	9	17	30	14·8	10·88	13·06	3·92
Sample B	9	20	35	14·9	10·50	12·69	4·4
Dairy meal	9	32	64	6·48	5·50	5·98	0·98

The Regulations allow for a variation of one-eighth above and below the guarantee fibre content; this allowance covers the variation from the average with the bran samples and dairy meal, but not with the extracted palm kernel meal, where 10 per cent. of the results were outside the permissible limits for sample A and 15 per cent. for sample B. The spread between the highest and lowest figures is far too great for the method of estimation to be considered satisfactory, and further tests were designed to throw light on the possible causes of the variations.

A STUDY OF FACTORS AFFECTING THE ACCURACY OF FIBRE ESTIMATIONS—

In this estimation the sample has to be boiled with aqueous acid and alkali of specified concentration for a definite time, the rate of boiling being defined as "gentle and continuous." This definition may be interpreted in widely different ways. The results of varying the conditions and overstepping the boiling are given in Table XII.

In number (1) test, by the official procedure, an electric heater was used, according to our normal custom. The thoroughness of the agitation was determined by the vigour of

TABLE XII

Variation in boiling procedure	Fibre content of palm kernel cake %
(1) Official procedure	10·4
(2) Vigorous boiling	10·8
(3) Simmering over a bunsen flame	12·8
(4) Simmering in a boiling water-bath	19·2
(5) Acid boil of 1 hour	8·8
(6) Alkali boil of 1 hour	9·1
(7) Acid and alkali boils of 1 hour each	7·4

the boiling and it will be seen that as ebullition became milder, the fibre figure increased. In the official method, the acid and alkaline boiling periods are specifically limited to exactly 30 minutes, but if this time were extended by oversight, it would lead to lower fibre figures, as shown in the above table.

During vigorous boiling, the foam tends to deposit solid particles in the flask outside the area of the reaction, and experiment showed that the use of an air tube, supplying a gentle and steady jet of air from a mechanical blower on to the surface of the boiling liquid, prevented bad frothing and washing of the meal above the general liquor level; this device gave more consistent figures for palm kernel meal than the normal procedure, and it is mentioned in an American publication.[4] Ebullition, as a means of controlling temperature and agitation in a heterogeneous reaction, cannot be considered satisfactory and it is suggested that mechanical agitation with a suitable stirrer would make for much more consistent results.

To determine the effect of fineness of grinding, a sample of palm kernel cake was ground in the normal way to pass a B.S.S. No. 16 mesh sieve (apperture 1/25 inch or 1 mm.). Part of this ground sample was further ground to pass a sieve having an apperture of 1/64 inch, or 0·4 mm. Fibre was estimated on each of these portions, using both 2 and 3 g. (Table XIII).

TABLE XIII

EFFECT OF FINENESS OF MEAL ON FIBRE ESTIMATION

Feeding stuff	Number of labs.	Number of operators	Number of tests	Fibre, % Highest	Lowest	Average
Palm kernel cake:						
Ordinary powder: 2 g.	9	24	44	14·6	10·0	11·7
3 g.	9	24	44	14·1	10·1	12·2
Fine powder: 2 g.	9	24	44	13·0	9·6	10·8
3 g.	9	24	44	13·5	9·4	11·5

On applying the regulation allowance of one-eighth above and below the average figure, it was found that 23 per cent. of the figures obtained with 2 g. of ordinary powder were outside the permissible limits, and 14 per cent. of the figures on 3 g. of ordinary powder. Similarly for the fine powder, 11 per cent. of the figures fell outside the permissible limits with 2 g. and 16 per cent. with 3 g.

The finer powder gave significantly lower fibre figures. A study of variance by statistical methods suggests that this is due not to mechanical loss of meal during filtration but rather to a more ordered reaction between the powder and the liquids and to easier manipulation in the flask. The variance factor for the different laboratories suggested that they differed somewhat in their technique and also there was a very significant difference between operators, some obtaining consistently high and others consistently low figures.

The results of these tests suggest that the official method requires tightening up, or modification, in the following respects—

The fineness of the sample and the weight to be taken should be stated without an alternative. The method of agitation should be improved. In addition, the method of drying after oil extraction should be specified and it is recommended that a reflux condenser should be attached to the conical flask during the boiling.

Nine oil-mill laboratories of Lever Brothers and Unilever Limited have taken part in this work.

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THE BRITISH OIL AND CAKE MILLS LIMITED
STONEFERRY, HULL

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The Chemical Estimation of Nicotinic Acid, using *p*-Aminopropiophenone

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PREVIOUS workers[1,2] have shown that with various foods satisfactory agreement can be obtained between microbiological and chemical assays of nicotinic acid, the latter by the cyanogen bromide method. The aromatic amines used have included metol, aniline, procaine and *p*-aminoacetophenone (PAAP). Of these it was found[2] that *p*-aminoacetophenone was the most satisfactory for examining certain cereal products of low nicotinic acid content, since it produced under the specified experimental conditions the greatest intensity of colour with a given amount of nicotinic acid. However, with some foods, especially low-extraction wheat flours, maize and oats, marked discrepancies occurred, the chemical method giving considerably higher results than the microbiological method. After we had begun to investigate this problem, supplies of *p*-aminoacetophenone failed, and an alternative aromatic amine had to be found. Procaine did not prove satisfactory, and metol and aniline gave too feeble colours. We then tried *p*-aminopropiophenone (PAPP), and found this to give results equal to those given by *p*-aminoacetophenone. This paper describes our findings with the new amine, and also shows how discrepancies between chemical and microbiological results can be largely eliminated by using a more complete set of blanks.

CHEMICAL METHOD

REAGENTS—

p-Aminopropiophenone (*PAPP*)—Commercial samples were found to require purification, being strongly coloured, almost insoluble in water and only partly soluble in alcohol. They should be extracted with several lots of ether, and the pooled ether extracts decolorised with "charcoal for adsorption." After filtration, the ether should be removed and the purified amine recrystallised several times from alcohol. Solutions should be freshly prepared each day, by dissolving 1·25 g. in a mixture of 1·76 ml. of concentrated hydrochloric acid, 17·5 ml. of alcohol and water to make up the volume to 25 ml. These solutions should be kept in brown bottles in the dark. (The quantity of acid was chosen to give the most suitable degree of acidity in the final reaction mixture.)

The other reagents required have been described previously.

PREPARATION OF EXTRACT—

This is carried out as in the previous method,[2] except that in the preparation of the "final extract" the volume should be made up to 25 ml. *not* with water but with alcohol. If any precipitation occurs, centrifuge and use the supernatant liquid.

DEVELOPMENT OF COLOUR—

Prepare the following mixtures (in ml.)—

	U	U + NA	SB	TRB	AB	ARB	CB	CRB
Final extract	3	3	3	—	3	—	3	—
Standard nicotinic acid solution	—	1	—	—	—	—	—	—
Alcohol	3	2	7	6	5	8	4	7
Timing now becomes critical. At known times add—								
CNBr	2	2	—	2	—	—	2	2

Put in water-bath at 56° to 58° C. for 4 minutes and then in water-bath at 20° C. for 5 minutes in the dark. Then add—

Alcohol	—	—	—	—	—	—	1	1
Mixed amine reagent	2	2	—	2	2	2	—	—
Total volume	10	10	10	10	10	10	10	10

Keep the mixtures in the dark, and at 4½, 5 and 5½ minutes after adding the mixed amine reagent measure their optical densities against water, according to a timetable as suggested below (in minutes).

	U	U + NA	CB	CRB	U	U + NA
Add CNBr and place in water-bath at 56° to 58° C.	0	3	7	10	13	16
Transfer to water-bath at 20° C. in dark	4	7	11	14	17	20
Take out of bath	8½	11½	15½	18½	21½	24½
Add amine reagent, mix well and put in cuvettes.						
Take measurements of optical density as follows—						
1st reading	13½	16½	20½	23½	26½	29½
2nd reading	14	17	21	24	27	30
3rd reading	14½	17½	21½	24½	27½	30½

This allows for duplicates of U and U + NA. Duplicates of CB and CRB can be added if desired, also duplicates of other blanks, for which timing is not critical.

Optical measurements—

As described previously.[2]

Calculation—

Let the mean readings of the respective tubes be—

U	for final extract of sample.
U + NA	for final extract of sample to 3 ml. of which 10 µg. of nicotinic acid have been added.
TRB	for total reagent blank.
SB	for solution or test blank.
CB	for cyanide or cyanogen bromide blank.
CRB	for cyanide reagent blank.
AB	for amine blank.
ARB	for amine reagent blank.

Then the nicotinic acid content of the sample is—

$$\frac{\mathrm{U} - \mathrm{TRB} - [(\mathrm{AB} - \mathrm{ARB}) + (\mathrm{CB} - \mathrm{CRB}) - \mathrm{SB}]}{(\mathrm{U} + \mathrm{NA}) - \mathrm{U}} \times \frac{25}{5^{*}} \times \frac{20}{3} \times \frac{\text{vol. of eluate}}{\text{vol. of sample used}}\ \mu\text{g./g.}$$

* Assuming the measured volume of the supernatant liquid used in preparation of the final extract was 5 ml., as given in the directions. If it was not, the appropriate correction must be made.

which simplifies to—

$$\frac{\mathrm{U} - \mathrm{TRB} - \mathrm{AB} + \mathrm{ARB} - \mathrm{CB} + \mathrm{CRB} + \mathrm{SB}}{(\mathrm{U} + \mathrm{NA}) - \mathrm{U}} \times \frac{100}{3^{*}} \times \frac{\text{vol. of eluate}}{\text{vol. of sample used}}\ \mu\text{g./g.}$$

Microbiological method

The microbiological assay of nicotinic acid using *L. arabinosus* 17/5 is now well known, and does not call for detailed description. The method adopted in these studies is almost exactly as described and recommended by the Microbiological Panel of the Vitamin Estimations Sub-Committee of the Society of Public Analysts and Other Analytical Chemists.[3]

The possibility that the organism is sensitive to fats and fatty acids has been recently confirmed (Lynes and Norris[4]). In order to avoid complications of this kind, some of the samples were pre-extracted with light petroleum to remove fat. In all cases, however, the final extract was shaken with light petroleum prior to filling the assay tubes.

The results quoted are the mean of a number of assays, each at three or four levels of dosage. The calculation is made by reference to the equation to the standard curve of best fit. Confirmatory calculations based on the ratio of the slopes of calculated standard and sample curves were also made. Only results of assays of undoubted validity have been included in compiling Table I. The accuracy of a single determination by microbiological methods has somewhat arbitrarily been regarded as ±10 per cent.; but with careful working greater accuracy may be expected, and in the present series the figure has been reduced, on average, to about ±5 per cent. This limitation is of some importance when considering the closeness of agreement of results by microbiological and by chemical methods.

TABLE I

ASSAY OF NICOTINIC ACID BY MICROBIOLOGICAL METHOD

Results in μg. per g. of original material

Sample assayed	Content	Standard error of determination	Coefficient of variation
Barley ..	73·5	2·6	3·5
Malted barley	82·9	2·9	3·5
Malt and oil	84·8*		
Malt extract	94·8	4·9	5·2
Malt and yeast food	91·2	7·1	7·8
Dried yeast	329·0	15·6	4·7
Wheat, whole	50·5	3·4	6·7
Wheat flour, 80% extraction	17·5	0·4	2·3
Wheat flour, fortified	51·0	3·0	5·9
Wheat, germinated	57·5	2·0	3·4
Yeast extract A	540·0	21·9	4·1
Yeast extract B	488·0	17·4	3·6

* Average of results previously published.

The above was written before publication of a paper by Kodicek and Pepper.[5] Detailed comment on their findings in respect of the microbiological assay of nicotinic acid is inappropriate in this communication. It may be mentioned, however, that many variations in the composition of the medium were tested by the Microbiological Panel, and the most suitable was that finally published. Subsequent use of this medium in a large number of assays would seem to support the conclusions of the Panel. It is agreed that the somewhat optimistic estimates of coefficients of variation anticipated by some workers have not been justified; but we have, in general, found lower coefficients than those indicated by Kodicek and Pepper. Where the variation has been high it has always been found that this is due to the inclusion of assays which, for various reasons, were invalid.

SPECTROSCOPIC STUDY OF COLOUR GIVEN BY DIFFERENT AMINES

The selection of the secondary filter used in the photo-electric estimation of the colour given by nicotinic acid with the amine and cyanogen bromide should be based on the absorption curve of the colour. We determined this, using the Beckman Model DU Spectrophotometer, on a series of freshly prepared colours, the average reading at each wavelength being measured at the same number of minutes after mixing, so that allowance was made for any errors that might have arisen from the instability of the colour. The curves obtained by using *p*-aminopropiophenone and *p*-aminoacetophenone were determined separately. The results, plotted in Fig. 1, showed, with *p*-aminopropiophenone a rather broad band with a peak at about 445 mμ., very similar to that given by *p*-aminoacetophenone, which has a peak at about 455 mμ. No significant shifts occurred in these maxima during the time taken to carry out an assay, although the densities were of course changing. The peak given by *p*-aminoacetophenone at 455 mμ. is not shown in the extinction curve of the *p*-aminoacetophenone colour published by Kodicek[6] in 1940 and obtained with the Zeiss step-photometer using filters which provided only a rough indication of the curve. It was, however, found by Dann and Handler[7] (at about 440 mμ.) using the Coleman DM Spectrophotometer. These spectroscopic data are summarised in Table II; and show that the No. 7 Chance's violet filter, with maximum transmission at about 440 mμ., is equally suitable when using *p*-aminoacetophenone, *p*-aminopropiophenone or aniline, but less satisfactory when using metol as the aromatic amine.

TABLE II

SPECTROSCOPIC DATA ON COLOURS GIVEN BY DIFFERENT AMINES IN CNBr TEST FOR NICOTINIC ACID

	Absorption maxima (in mμ.) obtained with			
	Metol	Aniline	PAAP	PAPP
Dann and Handler	400	435	440	—
Klatzkin, Norris and Wokes ..	—	—	455	445

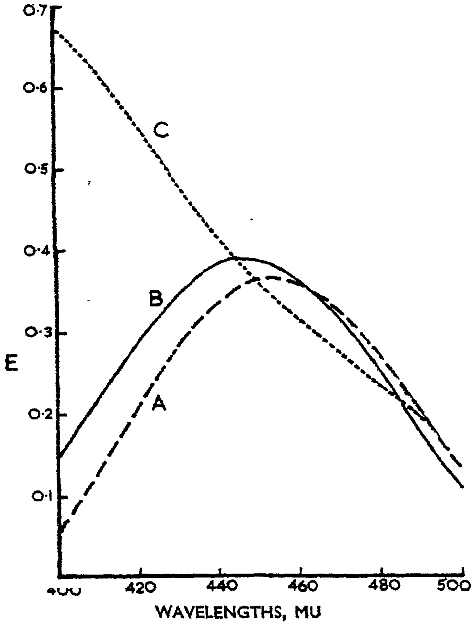

Fig. 1. Absorption curves of yellow colour given by nicotinic acid with CNBr and PAAP or PAPP

Curve A. PAAP (at 2–4 min. after mixing) against TRB

Curve B. PAPP (at 1¼–4¼ min. after mixing) against TRB

Curve C. PAPP (at 1¼–4¼ min. after mixing) against alcohol

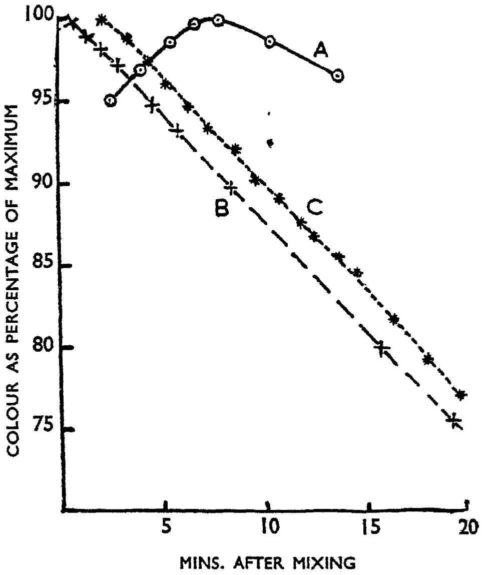

Fig. 2. Rates of development and fading of colour given by nicotinic acid with CNBr and PAAP or PAPP

Curve A. PAAP in alcoholic solution
" B. PAAP in aqueous solution
" C. PAPP in alcoholic solution

RATE OF FADING OF COLOUR—Readings of the colour obtained when using *p*-aminopropiophenone as aromatic amine, under the given experimental conditions, were taken with the Spekker absorptiometer at different times after mixing. The results of a number of experiments are plotted in Fig. 2. They show that the rate of fading of the colour resembled that previously observed with *p*-aminoacetophenone in aqueous solution, but was more rapid than was given by that amine in alcoholic solution. The need for careful timing therefore applies equally to both amines if satisfactory results are to be obtained. It is of interest to note that the presence of alcohol in the reaction mixture retarded the development of the colour, so that the maximum was not reached until about 3 minutes after mixing, whereas in aqueous solution it was reached in less than a minute. A similar effect of alcohol was previously observed when using *p*-aminoacetophenone.

CALIBRATION OF ABSORPTIOMETER—Table III summarises our results when calibrating the absorptiometer with pure nicotinic acid, using *p*-aminopropiophenone under the given experimental conditions. They show that Beer's law is obeyed over the range 5 to 40 μg. per 10 ml., which amply covers the working range. The accuracy of the results compares favourably with that previously found with *p*-aminoacetophenone.

RECOVERY EXPERIMENTS WITH ADDED NICOTINIC ACID—

These were carried out with *p*-aminopropiophenone in the same manner as they had been applied to *p*-aminoacetophenone, and gave satisfactory percentage recoveries, ranging from 97 to 105 and averaging 101 in 130 assays on 34 different samples. This compares favourably with the percentage recoveries of 94 to 102 previously obtained with *p*-aminoacetophenone in 157 assays. We also obtained satisfactory recoveries when adding a known amount of nicotinic acid to the food before extraction in order to test the behaviour of the Lloyd's reagent.

TABLE III

CALIBRATION OF ABSORPTIOMETER WITH PURE NICOTINIC ACID USING *p*-AMINOPROPIOPHENONE

Concentration of NA μg. in 10 ml.	Mean optimum density (D) at 5 min. after mixing	$\frac{D \times 1000}{\text{concentration}}$
5	0·105	21·0
10	0·221	22·1
20	0·434	21·7
30	0·629	21·0
40	0·846	21·2
	Mean	21·4
	Standard error of mean	0·22

EXPERIMENTS ON VARYING PROPORTION OF ALCOHOL AND OF AMINE IN REACTION MIXTURE—

The maximum solubility of *p*-aminopropiophenone in alcohol at room temperature was about 1 in 60 or 1·5 per cent. By using 5 ml. of such a solution in 15 ml. of reaction mixture we obtained a final concentration of 0·5 per cent. of *p*-aminopropiophenone, giving with 20 μg. of nicotinic acid an absorptiometer reading of about 0·35 under the given experimental conditions. The amine could be rendered more soluble by adding to the alcohol the 6 per cent. hydrochloric acid used to obtain the necessary degree of acidity in the reaction mixture. We were thus able to prepare a 5 per cent. solution of *p*-aminopropiophenone, and by using 2 ml. of this in 10 ml. of reaction mixture obtained in the latter a final concentration of 1·0 per cent. of the amine. This gave, with 20 μg. of nicotinic acid, an absorptiometer reading of about 0·42. Thus, an increase of 100 per cent. in the concentration of amine gave an increase of only about 20 per cent. in the intensity of colour. We decided that even if the concentration of amine in the reaction mixture could have been further increased by using some other solvent, any resulting further increase in colour intensity would not have been of practical significance. When we came to apply this technique to foods we found that on mixing the alcoholic reagent with an aqueous extract of the food there might be gradual precipitation not detected until colour measurements had begun, when it was too late to make any correction. This difficulty was overcome by using alcoholic extracts of the food. Any opalescence appearing in these was removed by centrifuging before developing the characteristic colour. Fifty per cent. alcohol in the reaction mixture proved less satisfactory than about 80 per cent., the concentration finally adopted.

EFFECT OF pH—

The pH for adsorption and elution is of course critical, as shown by numerous workers. The effect of the pH of the reaction mixture, although equally critical, seems to have received very little attention. Previous work in these laboratories had shown that for *p*-aminoacetophenone and for procaine the optimal pH for the reaction is below 1, the colour intensity falling rapidly as the pH rises above 2, and reaching a minimum between 4 and 5. Our experiments with *p*-aminopropiophenone gave similar results (see Fig. 3). We therefore adopted for this amine the same reaction pH as for *p*-aminoacetophenone.

PREPARATION AND PURIFICATION OF EXTRACT—

The food material (in No. 30 or 40 powder) should be extracted with 4 *N* hydrochloric acid for 40 minutes in a boiling water-bath, as previously described.[2] In general, the findings of Kodicek and Pepper[5] confirm our belief that acid extraction is preferable to alkaline extraction, but we have obtained better results with slightly stronger acid than theirs. Alkaline extraction of cereals involves the production of greater amounts of interfering substances, and this is more marked where germinated cereals are under test. Our results for wheat, using acid extraction and amine blank, agree with those of Kodicek and Pepper, close concordancy between microbiological and chemical assays being obtained. We differ from them in that we do not obtain similar concordancy with ungerminated maize and oats.

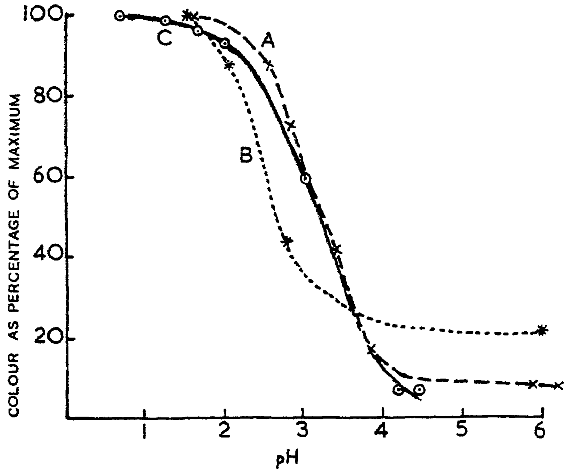

Fig. 3. Effect of pH on intensity of colour given with different amines in the cyanogen bromide test for nicotinic acid
Curve A. Results with PAAP
" B. Results with PAPP
" C. Results with procaine

It has been shown[2] that the use of potassium permanganate to remove interfering colours may lead to varying results, perhaps because of the development of slight haziness scarcely detectable visually but exerting a marked effect on the photo-electric cell. We have also found decolorisation with peroxide to be unsatisfactory and have obtained the most satisfactory results by adsorbing the nicotinic acid on Lloyd's reagent at an acid pH, eluting it with alkali and removing further interfering colour by adsorption on freshly prepared lead hydroxide as previously described.

ALLOWANCE FOR INTERFERING SUBSTANCES

These substances, which are incompletely removed by the purification processes, include—

(*a*) natural pigments;
(*b*) "amine chromogens"—developing colour with the aromatic amine;
(*c*) "cyanide chromogens"—developing colour with the cyanogen bromide.

In addition, allowance must be made for—

(*d*) the colour of the reagent; and
(*e*) the bleaching effect of the cyanide on certain colours.

Various blanks have been introduced to correct for these disturbing factors, *e.g.*, "total reagent blank" (TRB) for (*d*),[8] cyanide blank (CB) for (*a*) + (*c*),[9] amine blank (AB) for (*a*) + (*b*).[10] The bleaching effect of the cyanide may be diminished by adding acid immediately before adding the cyanide[11] in a special "acid blank" applied to urine, but this does not necessarily effect complete correction when applied to foods. More accurate allowance for the bleaching effect can be made by adding to the total colour, U, the solution blank plus cyanide reagent blank minus cyanide blank (SB + CRB − CB). This quantity is usually positive, hence correction for "bleaching" involves an *increase* in the colour measured, whereas correction for amine chromogens involves a *decrease*. With certain food materials the magnitude of the bleaching effect is similar to that of the amine chromogens, so that these two factors more or less counterbalance each other, and may both be ignored without seriously affecting the result. With some other food materials, these two factors cannot safely be neglected, and the complete set of blanks should be employed, as given above under "Chemical Method."

The magnitude of these different blanks is indicated by data obtained in 39 assays on various foods, using p-aminopropiophenone, and summarised in Table IV. The amine blank, AB, is seen to be the chief offender, providing 30 to 65 per cent. of the total colour measured. About half of AB is due to the amine, as can be seen by deducting from AB the blank ARB. The difference between AB and ARB was always greater than the solution blank, SB, the extra colour due to amine chromogens ranging from 6 to 19 per cent. of the total colour. Since the total reagent blank did not vary significantly from the sum of ARB and CRB there was little change in colour when mixing the two halves of the reagent. On the other hand, the bleaching effect of the cyanogen bromide was sometimes quite marked, SB + CRB − CB ranging from 3 to 13 per cent. of the total colour.

TABLE IV

MAGNITUDE OF BLANKS IN CHEMICAL ASSAYS OF NICOTINIC ACID USING PAPP

All results given as percentage which the given blank represents of the total colour given by final extract with reagent

Material	No. of assays	AB	ARB	AB − ARB	SB	AB − ARB − SB (amine chromogens)	CB	CRB	TRB	SB* + CRB − CB
Wheat, 80% extraction	6	65	35	30	11	19	5	7	42	13
Wheat, whole	3	56	36	20	12	8	7	6	38	11
Barley	4	44	26	18	7	11	5	5	27	7
Malted barley	4	46	27	19	8	11	6	4	32	6
Malt extract preparations	16	49	26	23	6	17	4	4	29	6
Yeast preparations	6	30	21	9	3	6	3	3	25	3

* Bleaching effect of cyanide.

COMPARISON OF METHODS USING DIFFERENT BLANKS—

Table V shows the wide deviation in results that may be obtained on a given sample by using different blanks. For facilitating comparison, the results are all expressed as percentage of the mean microbiological result. They show that the more completely allowance is made for interfering substances by means of blanks, the nearer the chemical results come to the microbiological results.

TABLE V

CHEMICAL ESTIMATION OF NICOTINIC ACID USING BLANKS INCLUDED IN DIFFERENT METHODS

All results quoted as percentage of mean microbiological result

Material	Results using blanks employed by				
	Bandier and Hald	Bandier	Harris and Raymond	Dann and Handler	Friedemann and Frazier
Barley 41	127	114	97	110	109
Malted barley 42	138	123	102	123	111
Malt and oil	133	133	104	122	104
Malt extract	128	128	100	122	96
Malt and yeast	119	119	116	111	111
Dried yeast	129	129	110	121	101
Wheat, whole	129	112	96	107	111
Wheat flour, 80% extraction ..	150	126	84	108	114
Wheat flour, fortified	113	97	76	87	90
Wheat, germinated	118	105	79	91	102
Yeast extract A	122	122	105	116	102
Yeast extract B	109	102	112	103	110
Means	129	118	98	110	105
Standard error of mean	± 3·2	3·3	3·7	3·4	2·1

Taking into consideration the combined errors of the chemical and microbiological methods, it may be assumed that on an individual sample a deviation of 20 per cent. between the mean results given by the two methods is probably significant, and on the average for the 12 samples included in the table a deviation of 10 per cent. between the results given by the two methods is almost certainly significant. When using Bandier and Hald's blanks, the chemical results on 8 out of 12 of the samples significantly exceeded the microbiological results, and a highly significant difference was also observed for the mean of all samples. With Bandier's blanks, the chemical results on 6 out of 12 of the samples significantly exceeded the microbiological results, and a significant difference was also observed for the mean of all samples. With Harris and Raymond's blanks, the chemical results on 2 out of 12 of the samples were significantly lower than the microbiological results, but the average for all samples did not differ significantly from the microbiological result. The latter was also true when using the blanks of Dann and Handler[7] or of Friedemann and Frazier.[12] Hence, the blanks used by Bandier and Hald, and by Bandier, give less satisfactory results than those used by the other workers mentioned in the table. For many types of foodstuffs the blanks of Harris and Raymond and of Dann and Handler will probably give as reliable and satisfactory results as the more complete set of blanks used by Friedemann and Frazier. However, with certain foodstuffs (*e.g.*, low-extraction wheat flour, oats and maize) discrepancies between chemical and microbiological results may arise, which can be markedly reduced by the use of the full set of blanks. There is, of course, the possibility that the discrepancy between chemical and microbiological results may, in certain instances, be due to the occurrence of an anti-vitamin diminishing the microbiological activity without affecting the chemical results, or alternatively the presence of a nicotinic acid precursor giving a positive response to the chemical but not the microbiological assay. This raises important questions which must be left over for future consideration. These questions do not seem likely to arise with the materials examined in the present investigation.

COMPARISON OF PAAP AND PAPP RESULTS—

In assays on 24 samples of cereals and cereal products the mean results with *p*-aminopropiophenone were 99 per cent. and ranged from 82 to 110 per cent. of the results with *p*-aminoacetophenone when examining the same sample. Two of these samples were a barley and a malted barley, which were the first we examined using the full series of blanks with *p*-aminopropiophenone, and gave results 82 and 86 per cent. of those obtained with *p*-aminoacetophenone. Omitting these 2 samples, the results on the remaining 22 gave mean results with *p*-aminopropiophenone ranging from 94 to 110 per cent. and averaging 100 per cent. of the results with *p*-aminoacetophenone.

COMPARISON OF PAPP WITH MICROBIOLOGICAL RESULTS—

The data in Table V showed that when using *p*-aminopropiophenone with a full set of blanks the average chemical result on 12 samples was 105 per cent. of the average microbiological result. Equally good agreement was obtained with other samples examined by both methods (for example, the nicotinic acid content of a barley sample was 98 μg. per g. chemically and 96 μg. per g. microbiologically). We therefore think that when estimating nicotinic acid in the types of material included in Table V the chemical method using *p*-aminopropiophenone with, if necessary, the full set of blanks, can be relied upon to give satisfactory results.

We are indebted to Miss Janet Horsford and Mr. R. Evans for assistance. The chemical work in this paper will form part of a thesis to be submitted for a Ph.D. in the University of London.

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January, 1949

Colorimetric Determination of Streptomycin B (Mannosido-streptomycin)

By W. B. EMERY AND A. D. WALKER

(Read at the meeting of the Society on Wednesday, May 4th, 1949)

No method has so far been published for the direct determination of streptomycin B (a mannoside of streptomycin, isolated by Titus and Fried[1]) in presence of streptomycin itself (which for convenience we shall call streptomycin A). By assuming a certain ratio (about 1 : 5) between the biological and chemical "potencies" of pure streptomycin B, it is possible to calculate the proportion of this antibiotic present in a sample of streptomycin. Since the ratio is not accurately known and will, of course, be constant only for a given strain of bacterium, no great trust can be put in results obtained in this way. The development of an accurate method of determining streptomycin B is described in this paper.

Morris[2] has reported the use of a 0·2 per cent. w/v solution of anthrone in 95 per cent. v/v sulphuric acid for the quantitative determination of carbohydrates. It seemed possible that this reagent might be applied to the assay of streptomycin B by determining the mannose residue in the molecule, always provided that the glucosamine and streptose moieties in the molecules of both streptomycins do not react with the reagent. Results obtained by the method described below have shown that they are not.

EXPERIMENTAL

Preparation of the reagent—Cautiously add 950 ml. of AnalaR concentrated sulphuric acid to 50 ml. of cold distilled water. It is essential that AnalaR sulphuric acid be used, since solutions of anthrone in commercial acid deteriorate rapidly. Cool the 95 per cent. sulphuric acid to room temperature and dissolve in it 2 g. of recrystallised anthrone, m.p. 153° to 154° C. (prepared as described by Morris[2]). The reagent is stored at 0° C. and is stable for at least a fortnight.

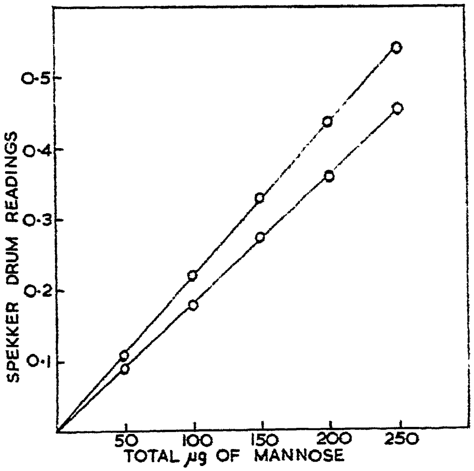

Fig. 1. Typical standard curves

Construction of a standard curve—Prepare solutions of pure mannose containing 10, 20, 30, 40, 50, 60, 80 and 100 µg. per ml. Add from a burette 10 ml. of the reagent to 5 ml. of each of these solutions, contained in 6 × 1 inch boiling tubes, which should be of uniform wall-thickness and diameter. Prepare a blank by adding 10 ml. of the reagent to 5 ml. of water. Gently shake the tubes to insure thorough mixing of the reagent with the solution, and after at least 20 minutes measure the absorption of the green solutions against the blank by means of a Spekker photo-electric absorptiometer, using Chance glass OR2 red filters (peak transmission 640 mμ.) and cells 2 cm. long. Plot the readings obtained against the number of micrograms of mannose in the 5 ml. of solution. The curve so constructed is a straight line passing through zero for absorptiometer readings up to at least 0·6, but occasionally flattens out at higher levels. The slope of the line varies slightly from day to day. Two typical curves are shown in Fig. 1.

ASSAY PROCEDURE AND CALCULATION OF RESULTS—

Dilute a solution of the streptomycin sample to be assayed until the concentration of streptomycin B is between 60 and 200 units per ml., corresponding to about 20 to 60 μg. of mannose per ml. (see calculation below). Add 10 ml. of reagent to 5 ml. of the solution and read the resultant yellowish-green solution against the blank of water plus reagent in the manner described above. Find the concentration of mannose (in μg. per ml.) in the streptomycin solution by reference to the standard curve. It is usual to assay the streptomycin solution at two different levels and average the results. Thus, one might take 2 and 4 ml. respectively of the solution, making each up to 5 ml. with water before assaying. The total streptomycin (in units per ml.) present in the test solution is determined by chemical assay based on the maltol - ferric ion reaction.[3,4] The molecular proportion of streptomycin B (*i.e.*, the molecular percentage of the total streptomycin present) in the sample may be calculated as follows.

Let a solution of streptomycin at X chemical units per ml. contain Z μg. of mannose per ml.

Then, since the molecular weight of mannose = 180
and the molecular weight of streptomycin B base = 743,

$$\text{the concentration of streptomycin B base} = \frac{743\text{Z}}{180}\ \mu\text{g. per ml.} = \frac{743\text{Z}}{180{,}000}\ \text{mg. per ml.} \quad .. \quad (1)$$

The expression (1) can be used to calculate the weight percentage of streptomycin B in a solid sample of streptomycin.

By definition, 1 mg. of streptomycin A base ≡ 1000 chemical units, and as the molecular weight of streptomycin A base is 581,

$$\frac{743}{581}\ \text{mg. of streptomycin B base} \equiv 1000\ \text{chemical units} \quad .. \quad .. \quad .. \quad (2)$$

From (1) and (2),

$$\text{Concentration of streptomycin B} = \frac{743\text{Z}}{180{,}000} \times \frac{581}{743} \times 1000\ \text{chemical units per ml.}$$

$$= \frac{581\text{Z}}{180}\ \text{units per ml.}$$

$$\text{Hence molecular proportion of streptomycin B} = \frac{581\text{Z} \times 100}{180\,\text{X}} = \frac{323\text{Z}}{\text{X}}\,\%$$

RESULTS—

The method described above has been applied to a number of samples of streptomycin hydrochloride and streptomycin calcium chloride complex. Samples chosen have contained from 3 to 95 per cent. of streptomycin B. The hydrochlorides containing over 50 per cent. of streptomycin B were prepared from production residues by modifications of the method of Titus and Fried.[1] The results obtained on a few samples are recorded in Table I, together with the biological/chemical assay ratios, and the proportions of streptomycin B calculated from these ratios. Ratios of 0·2 and 1·0 for pure streptomycin B and streptomycin A respectively have been assumed.

It seemed desirable to demonstrate the reliability of our method in some way other than by comparison with results obtained as indirectly as those tabulated. Accordingly, we assayed a sample of streptomycin calcium chloride complex, recrystallised the sample and assayed the recrystallised material and the mother liquor. Of 330 mg. of streptomycin B present in the starting material (5 g.), 168 mg. were recovered in the mother liquors and 148 mg. in the recrystallised complex (96 per cent. recovery). This, in conjunction with the results above, appears to indicate that streptomycin B will form a calcium chloride complex, but that this salt is more soluble than that of streptomycin A.

TABLE I

Sample No.	Type of compound	Assay ratio	Molecular proportion of streptomycin B	
			Calculated from ratio	By anthrone assay
1	Hydrochloride	0·240	95	90
2	,,	0·240	95	97
3	,,	0·245	94	94
4	,,	0·560	55	54
5	,,	0·592	51	54·5
6	,,	0·760	30	40·5
7	,,	0·620	47·5	43·0
8	,,	0·720	35·0	39·0
9	,,	0·760	30·0	28·5
10	$CaCl_2$ complex	1·000	0·0	3·6
11	,,	0·930	8·8	7·1
12	,,	0·870	16·3	9·5
13	,,	0·880	14·8	9·1

SUMMARY—

The use of Morris's reagent (0·2 per cent. of anthrone in 95 per cent. sulphuric acid) for the quantitative estimation of streptomycin B is described. Results obtained by the method given are in accord with those calculated from chemical and biological assays, assuming the relative biological activities of the two streptomycins are as stated.

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August, 1948

DISCUSSION

The **PRESIDENT** congratulated the authors on their interesting contribution on what was to most present a new problem. He asked what were the relative antibiotic values of streptomycins A and B and what was the nature of anthrone.

Dr. J. H. **HAMENCE** enquired if the method could be applied to soils or soil extracts; he was interested to know what means were available for investigating the antibiotic substances in soils.

Mr. **EMERY**, replying to the President, said that in terms of activity towards *B. subtilis*, streptomycin A was about five times as potent as streptomycin B. Anthrone was partially reduced anthraquinone. Streptomycin A did not respond to the test described to any appreciable extent.

Mr. **WALKER** said that in soils there might be many substances other than antibiotics that would react with anthrone, but few that would react to both the anthrone and the maltol - ferric ion tests.

The Reduction of Antimonial Tin Solutions with Metallic Nickel and Cobalt

BY H. HOLNESS

(Read at the meeting of the Society on Wednesday, May 4th, 1949)

THE reduction of tin solutions containing antimony by means of metallic iron was studied by Järvinen[1] who showed that the precipitated antimony co-precipitated appreciable amounts of tin. More recently, Evans and Higgs[2] studied the use of metallic lead, iron, nickel, aluminium and zinc for the same purpose and reported that "only nickel causes no co-precipitation of tin with either copper or antimony." As a result, Schoeller and Holness[3] used nickel powder to remove antimony in their gravimetric determination of tin in alloys by the tannin method and in furtherance of this work I have tried to apply it to the determination of minor amounts of tin in antimony regulus.

It was in this connection that the protective action on nickel of strong hydrochloric acid solutions of antimony trichloride was encountered, an effect previously studied by Clarke[4] and more recently by Piontelli and Fagnani.[5] In both cases the conditions determined were those affording protection, not those causing a maximum attack on the nickel. In a recent paper[6] these latter conditions were established as requiring: (1) an acid concentration of about 2·5 *N*, (2) heating to be carried out on a water-bath, and (3) the solution on no account to be boiled or mechanically agitated.

It should be pointed out that Evans and Higgs reached the general conclusion quoted above, using only one concentration of antimony and tin, *viz.*, 0·2 g. of each in 100 ml. of acid. Their technique was to boil the solutions for 30 minutes with sheet nickel, to filter hot and to wash the precipitate thoroughly with hot, diluted hydrochloric acid (1 in 10) and "subsequently to test the metallic precipitate for tin and, if it is present, to determine its proportion in the bulk of the precipitate." No details are given, however, of the method used to determine this tin.

In the work recorded below, antimonial tin solutions were treated with nickel powder using a technique similar to that quoted above. The tin was determined in the filtrate and any negative error in recovery attributed to co-precipitation. The ratios of tin to antimony were of an order calculated to test critically the validity of the statement regarding co-precipitation by nickel.

There seems to be no record of the use of metallic cobalt as a reductor in tin determinations and since hydrochloric acid solutions of antimony behave towards cobalt in much the same way as towards nickel,[4] a few experiments were included to establish its behaviour so far as co-precipitation was concerned.

EXPERIMENTAL

Small amounts of tin were added to large concentrations of antimony to give ratios ranging from 1 to 0·1 per cent. of tin in antimony. After reduction with nickel powder the precipitated antimony was filtered and washed and the filtrate oxidised with bromine water. Iron in the form of ferric chloride was then added to act, first as a collector of the tin, and then as an indicator in the subsequent recovery of the tin by precipitation with tannin—a technique which had proved its worth in previous work.[3]

PROCEDURE—

Carefully weighed pieces of A.R. granulated tin were added to 1·0 g. lots of antimony metal powder ("ex tartar emetic") and each was placed in a 750-ml. conical flask and treated with 100 ml. of diluted hydrochloric acid (1 + 1) containing sufficient bromine to dissolve it. After solution the excess of bromine was removed by boiling and 100 ml. of water were added to reduce the acidity to about 2·5 *N*. Five g. of fine nickel powder were then added and the whole heated on a water-bath until reduction was complete—about 15 to 20 minutes—this stage was denoted by the evolution of *small* gas bubbles from the unused nickel, which continually forced to the surface of the liquid the black mass of nickel plus antimony. This mass was filtered through a pad of paper pulp sprinkled with a little nickel powder, and washed well with hot *N* hydrochloric acid. The filter pad with its precipitate was then transferred to a beaker and boiled with 100 ml. of *N* hydrochloric acid, filtered and again well washed. This technique of filtering and washing was observed in all the experiments using metal powders. The combined filtrates and washings were treated with a little bromine water to oxidise the tin to the stannic state, 5 to 10 drops of a 10 per cent. solution of ferric chloride, 5 g. of ammonium chloride and sufficient diluted aqueous ammonia (1 + 1) to make the solution just neutral to litmus. The solution was boiled, a little paper pulp added and the precipitated ferric hydroxide containing the stannic hydroxide filtered through a No. 41 Whatman filter. The filtrate was discarded and the precipitate washed with a hot 2 per cent. solution of ammonium chloride before being dissolved on the paper, in a hot solution of 5 g. of ammonium oxalate in 20 ml. of *N* hydrochloric acid. After the filter had been well washed with hot water, the filtrate and washings were treated with tannin and the tin determined by the method described in a previous paper.[3]

The first few experiments (Nos. 1 to 5) clearly showed a small co-precipitation of tin with the antimony and, in so far as conditions allowed, minor changes in technique were made with a view to the recovery of this small amount. Evans and Higgs carried out their reductions in boiling solutions, but this was only possible here by substantially increasing the bulk of liquid, owing to the protective action of the high antimony concentration. Compromises were therefore effected; in Experiment 6 the solution was boiled for 5 minutes after the reduction was complete and before filtration, and in Experiment 7 the precipitated metal, after filtration, was boiled with 1 g. of nickel powder in 100 ml. of 2·5 *N* hydrochloric acid for 5 minutes and filtered, and the filtrate and washings were added to the previous filtrate. These modifications in procedure however, brought about no change in the amount of tin co-precipitated and it was decided to conduct at least one experiment in boiling solution—Experiment 8. The antimony concentration was reduced by dilution with 2·5 *N* hydrochloric acid to a value equal to that used by Evans and Higgs, the nickel was added and the solution boiled for 30 minutes, after which the experiment was continued as before. It was noted that reduction under these conditions proceeded more slowly, but the result appeared to be the same.

TABLE I

Expt. No.	Antimony taken g.	Tin taken g.	Nickel used g.	Tin found g.	Error (tin co-pptd.) g.
1	1·0	0·0012	5	0·0007	− 0·0005
2	1·0	0·0052	5	0·0039	− 0·0013
3	1·0	0·0036	5	0·0027	− 0·0009
4	1·0	0·0065	5	0·0051	− 0·0014
5	1·0	0·0093	5	0·0077	− 0·0016
6	1·0	0·0019	5	0·0012	− 0·0007
7	1·0	0·0080	5 + 1	0·0065	− 0·0015
8	1·0	0·0042	5	0·0031	− 0·0011
9	1·0	0·0010	5	(*a*) 0·0005	
			5	(*b*) 0·0003	− 0·0002
10	1·0	0·0022	5	(*a*) 0·0013	
			5	(*b*) 0·0004	− 0·0005
11	1·0	0·0081	5	(*a*) 0·0061	
			5	(*b*) 0·0011	− 0·0009

Re-treatment of the precipitated antimony was next tried—Experiments 9 to 11. The precipitate was dissolved in hydrochloric acid and bromine, diluted and treated as before with nickel powder: a small recovery of tin resulted. This recovery furnished further proof that tin was co-precipitated to a small degree with nickel-reduced antimony.

It seemed reasonable to suppose that the co-precipitation of the tin would vary with the amount of antimony present, in which case the amount co-precipitated by the nickel-reduced antimony in the work of Evans and Higgs would have been too small to be apparent. Experiments 12 to 16 were designed to test this supposition. Equal aliquots of a standard solution of ammonium stannichloride were used and different weights of antimony trioxide added. The acidity was then adjusted to 2·5 *N* in hydrochloric acid and reduction effected with 5 g. of nickel powder as before. The results were surprising, for there was little or no alteration in the amount of co-precipitation, in spite of the fact that the amount of antimony used ranged from 1 to 0·1 g.

This unexpected result focussed attention on the method used to recover the tin after reduction, and alternative procedures were explored.

TABLE II

Expt. No.	Tin taken g.	Sb_2O_3 taken g.	Sb equiv. g.	SnO_2 found g.	Sn equiv. g.	Error (Sn co-pptd.) g.
12	0·0085	1·2	1·0	0·0089	0·0070	− 0·0015
13	0·0085	0·9	0·75	0·0088	0·0069	− 0·0016
14	0·0085	0·6	0·5	0·0090	0·0071	− 0·0014
15	0·0085	0·3	0·25	0·0090	0·0071	− 0·0014
16	0·0085	0·12	0·1	0·0092	0·0072	− 0·0013

Several methods of precipitating the tin together with the iron from a large concentration of nickel or cobalt offered themselves and four methods were examined. Using a stock solution of tin, 25-ml. portions were reduced for 5 minutes in hot 2·5 *N* hydrochloric acid solution with 5 g. of nickel powder, after which the unused metal was removed by filtration and well washed with hot *N* hydrochloric acid in the manner described above. The filtrate and washings were oxidised with bromine water and 5 to 10 drops of a 10 per cent. aqueous solution of ferric chloride added. The tin was then determined by each of the following methods:—

(*a*) Solid ammonium chloride was added followed by an excess of ammonia—sufficient to form the blue nickel hexammine. The solution was boiled and filtered, and the precipitate dissolved in 50 ml. of *N* hydrochloric acid containing 5 g. of ammonium oxalate, the tin being determined by tannin precipitation.

(*b*) Solid ammonium chloride was added and sufficient ammonia to render the solution just neutral to litmus. The solution was boiled and filtered and the precipitate treated as in (*a*).

(*c*) Solid ammonium chloride and 10 g. of ammonium acetate were added and the solution was neutralised to litmus with ammonia. It was then boiled and filtered and treated as in (*a*).

(*d*) Solid ammonium chloride and a solution of 1 g. of tannin in 25 ml. of water were added and the solution boiled. Ammonia was then added to the gently boiling solution until the purple colour of the iron tannin complex was just apparent; the precipitate was then filtered and treated as in (*a*).

The stock solution of tin, prepared by dissolving recrystallised ammonium stannichloride in *N* hydrochloric acid, was standardised by the tannin method and also by sulphide precipitation; 25 ml. gave 0·0517 g. and 0·0516 g. of SnO_2 respectively.

Two determinations with 25 ml. of the stock solution treated by method (*a*) gave only 0·0442 g. and 0·0466 g. of SnO_2. With methods (*b*), (*c*) and (*d*), however, 0·0503 g., 0·0505 g. and 0·0504 g. of SnO_2 respectively, were obtained. From these results two facts emerge: (i) the use of excess of ammonia must be avoided, as some ammine formation probably takes place, and (ii) the nickel itself would appear to remove a small amount of tin. This latter possibility was examined.

Portions of the stock solution of tin were added to 200 ml. of 2·5 *N* hydrochloric acid, boiled and treated with 5·0 g. of the nickel powder for 5 minutes. The powder was then removed by filtration and well washed as usual with hot *N* hydrochloric acid. The tin was determined in the filtrate by the method (*b*) described above. The unused metal together with the filter were boiled with 200 ml. of 2·5 *N* hydrochloric acid until all the metal had dissolved. The solution was filtered and the filtrate treated as above with a view to the recovery of any tin that may have become attached to the nickel. This was repeated with different amounts of nickel powder.

TABLE III

No.	Ni used	Tin taken SnO_2	Tin found: In filtrate SnO_2	Tin found: Recovered from Ni SnO_2	Total SnO_2	Error SnO_2
		g.	g.	g.	g.	g.
17	5 g.	0·0233	0·0224	0·0008	0·0232	− 0·0001
18	5 g.	0·0103	0·0088	0·0013	0·0101	− 0·0002
19	5 g.	0·0103	0·0091	0·0011	0·0102	− 0·0001
20	3 g.	0·0103	0·0095	0·0007	0·0102	− 0·0001
21	1 g.	0·0103	0·0100	0·0003	0·0103	0·0000
22	5 g.	nil	—	nil	—	—
23	12 sq. in.	0·0103	0·0103	nil	0·0103	0·0000
24	36 sq. in.	0·0233	0·0230	trace	0·0230	− 0·0003
25	36 sq. in.	nil	—	nil	—	—

The results of Experiments 17 to 21 show that there is a small retention of tin by the metallic nickel but the amount is less than a milligram. This small quantity does not wholly account for the errors noted in the previous experiments, and it must be concluded that these are composite errors, the loss of tin being caused partly by the unused nickel powder and partly by the precipitated antimony.

In view of these results and the widespread use of nickel as reductant in the volumetric determination of tin, Experiments 23 and 24 were carried out with thin sheet nickel in place of nickel powder.

It was realised that the fine nickel powder used in this work offered a very much larger surface area than could be conveniently obtained by the use of sheet nickel and it was to be expected that no really conclusive results would be obtained, but it is worth recording, that the *tendency* for the sheet nickel to retain tin is present, for with 36 square inches of surface, a trace of tin was detected. Any errors resulting from this tendency are usually compensated for in practice by the use of nickel-reduced tin solutions for the standardisation of the iodine. It is, however, of interest to note that in a recent paper, Miller and Currie tried to determine 10-mg. quantities of tin by iodine titration after nickel reduction and observed "a persistent negative error which occasionally reached 2·5 per cent."[7] Here the iodine solution was standardised by arsenious oxide, so that a part of their error could well be attributed to the retention of some tin by the nickel.

In general, this investigation has shown that some small loss of tin results from the use of nickel as a reductant, that the loss is increased if antimony is present, but that even then the amount is only very small. It does not invalidate the use of nickel in the volumetric determination of tin on the macro scale, but it would seem advisable to use nickel-reduced tin solutions to standardise the iodine solution. The use of nickel powder to remove antimony in the determination of tin in alloys by the tannin method[3] would appear to be permissible, only provided the amount of nickel powder used does not exceed 1 g. Where larger amounts are necessary, as in the determination of tin in antimony regulus, the precipitated metal must be dissolved and re-treated.

EXPERIMENTS WITH METALLIC COBALT—

It was assumed that much the same conditions would be required for the reduction of antimony solutions by metallic cobalt as were required by the nickel. The procedure followed, therefore, was that described above.

It was found that the cobalt was more vigorous than nickel in its action, and the reductions were complete in a much shorter time (from 3 to 5 minutes). The precipitated antimony, however, differed somewhat in character from that reduced by nickel, for it floated and made filtration tedious; with nickel the antimony had remained at the bottom of the flask except that it was carried to the surface from time to time by the accumulated gas; it was also possible to decant most of the liquid through the filter when nickel was used.

TABLE IV

Expt.	Antimony taken	Tin taken	Cobalt used	Tin found	Error (tin co-pptd.)
	g.	g.	g.	g.	g.
26	1·0	0·0043	5·0	0·0017	– 0·0026
27	1·0	0·0111	5·0	0·0051	– 0·0060
28	1·0	0·0163	5·0	0·0120	– 0·0043
29	1·0	0·0407	5·0	0·0321	– 0·0086

SUMMARY

The statement that only nickel causes no co-precipitation of tin with antimony, has been critically examined. Antimonial solutions containing from 1 to 0·1 per cent. of tin were reduced by nickel powder and small co-precipitations of tin were observed. These amounts were much smaller than those observed by Evans and Higgs using lead, iron, aluminium and zinc—they amounted at most to 1 part of tin in 500 parts of precipitated antimony.

The small losses of tin were caused partly by co-precipitation with the antimony and partly by retention on the surface of the remaining nickel powder. When sheet nickel was substituted for the powder, only a trace of retained tin was noted.

Experiments using metallic cobalt in place of nickel showed it to be less satisfactory.

Of all the metals used for the reduction of antimonial tin solutions it would appear that nickel causes the least co-precipitation of tin, with cobalt next, followed by iron, lead, aluminium and zinc in that order.

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January, 1949

DISCUSSION

Mr. F. L. OKELL said that the investigation would be of interest to chemists who have to deal with ores and alloys of tin containing antimony; for it substantiated the opinions of those who hold that the reduction by nickel foil of stannic chloride solutions containing antimony does not lead to low tin figures. In addition, the author has shown that when the precipitated antimony and the excess nickel powder is filtered on a pad sprinkled with nickel powder and washed with *N* hydrochloric acid, the filtrate is free from antimony. This adds a useful separation to the analytical chemistry of antimony.

Had the author, during his experiments with nickel as a reductant for tin, had any experience of inactive nickel that fails to effect complete reduction? This phenomenon, possibly caused by the formation of a protective film, had been reported many times, recently by Miller and Currie (1948). He had met with but one example, a low grade metal containing carbon and copper. Pure nickel, etched by boiling with hydrochloric acid and an oxidiser, chlorate, ferric chloride or bromine, gave complete reduction.

Dr. B. S. EVANS congratulated Mr. Holness on what seemed to be a valuable piece of work. It was no particular surprise to him to learn that there was, after all, evidence of a small co-precipitation of tin with the antimony thrown down by nickel. He had expected such co-precipitation and had been very perplexed when it did not seem to occur. A very notable occurrence, however, with the nickel they had used, was the smallness of the total precipitation of antimony, sometimes not much more than a trace; this he did not remember having heard mentioned by other workers.

There was one point that he thought should be mentioned, though it might well be a misunderstanding. In Table I the amount of tin co-precipitated would appear to be based on a difference figure and this after the main bulk had been boiled to dispel bromine. Tin in the presence of hydrobromic acid is very much more volatile than if hydrochloric acid alone is present and it might well be that at least some of the loss of about 1·5 milligrams might be put down to this cause.

With regard to Higgs's and his own work, he did not clearly remember the method they used to determine the co-precipitated tin in the antimony, but it was a *positive* method and was something like this— The washed precipitate was dissolved in bromine - hydrochloric acid, a large excess of oxalic acid added and the antimony removed by Clark's method by precipitation with hydrogen sulphide. The tin was recovered as stannous sulphide from the filtrate by a method he had published in 1932 (*Analyst*, 1932, **57**, 362), which at the time seemed to work very well.

Mr. HOLNESS, in reply to Mr. Okell, said that he had only met with inactive nickel when trying to reduce tin solutions containing antimony. In investigating this condition, he had come to the conclusion that, when antimony was present, it was wiser to activate the nickel by boiling with a hydrochloric acid solution of sodium chloride to which a very small amount of antimony trioxide had been added, rather than to use the method suggested by Mr. Okell. He could confirm that this method gave complete reduction with antimony-free tin solutions, but he had experienced trouble when antimony was present. He was of the opinion that nickel etched by hydrochloric acid and an oxidiser would become inactive in a more dilute antimony solution, than nickel activated by hydrochloric acid and sodium chloride containing a trace of antimony.

Referring to the possible loss of tin during the expulsion of bromine mentioned by Dr. Evans, he said that this had been envisaged, and in Experiments Nos. 12 to 16 (Table II) a standard tin solution was used together with antimony trioxide, in order to avoid the use of bromine. The results, however, showed a similar loss of tin to that obtained when bromine was used and the matter had not been pursued further.

ERRATUM: June (1949) issue, p. 351.

At the end of the first item in the list of References, *for* "540" *read* "54".

Notes

RAPID ESTIMATION OF FAT IN SAUSAGES AND SAUSAGE MEATS

THE following method was found to be very useful and gave results that agreed closely with those by the more standard Soxhlet method of fat extraction.

Place 10 ml. of sulphuric acid of sp.gr. 1·820 to 1·825 in a butyrometer. Thoroughly mince the sample of sausage (freed from casing) or sausage meat and place a representative portion on a watch glass, together with a small spatula. Weigh these, transfer about 2 g. of the sample carefully into the butyrometer and reweigh to ascertain the weight transferred. Introduce into the butyrometer also 1 ml. of amyl alcohol (of the standard required for the Gerber estimation of fat in milk) and sufficient water at approximately 80° C. to fill the butyrometer to the shoulder below the neck. Insert the stopper and carefully mix the contents of the tube by shaking until no more particles of meat are visible. Subsequently immerse in water at 68° C. and then centrifuge for 5 min. at about 1000 revs. per minute. At the end of this period return the tubes to the water-bath, where the readings are taken on the scale.

Multiply the percentage reading on the butyrometer by 11·2 and divide by the weight of sample taken, in grams, to obtain the percentage of fat in the sample.

Interference by the cereal content is overcome by immersion in boiling water, when the fat column rises above the cereal matter. The following results are typical of a number of experiments carried out on samples differing widely in fat content; the fat was determined both by Soxhlet extraction and by the above method.

By Soxhlet extraction	29·2	42·6	20·7	4·0	24·1	22·1 per cent.
By above method	29·7	42·8	20·85	3·2	24·3	22·4 per cent.

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September, 1948

THE DETERMINATION OF CAFFEINE IN COFFEE AND COFFEE PRODUCTS

The estimation of caffeine in coffee and coffee products by the method of Kidd, Nanji, and Edwards,[1] in which zinc ferrocyanide is used as a defecating agent, has been found in the authors' experience to give low or erratic results. This is particularly noticeable when the precipitate is allowed to stand for any length of time and especially overnight. An investigation of the method showed that the precipitate adsorbed caffeine on standing. The use of normal lead acetate in place of zinc ferrocyanide obviated this difficulty. The method finally adopted for this determination was as follows.

The infusion from ground coffee (concentrated if necessary) or the solution of coffee extract, or coffee and chicory essence, is transferred to a 100-ml. graduated flask and diluted to approximately 70 ml. and 5 ml. of 20 per cent. normal lead acetate solution are added slowly, dropwise, with shaking. The mixture is made up to the mark, mixed, and filtered. The filtrate is de-leaded by addition of a little solid powdered sodium oxalate and refiltered. Fifty ml. are then transferred to a separating funnel, 5 ml. of 0·880 aqueous ammonia are added and the caffeine is extracted with successive portions of chloroform, 40, 30, 20 and 10 ml. The combined chloroform extracts are washed with 10 ml. of *N* sodium hydroxide, the chloroform layer is separated and the caustic soda layer extracted with a further 15 ml. of chloroform. The chloroform is removed from the combined extracts by distillation and the residue of impure caffeine is dissolved in a small quantity of hot water and transferred to a 100-ml. Kjeldahl flask. The nitrogen determination is carried out in the normal manner, using 10 ml. of sulphuric acid, 5 to 10 g. of potassium sulphate and a crystal of copper sulphate.

With quantities of caffeine ranging from 10 to 70 mg. in coffee and coffee products and with aqueous solutions of pure caffeine, consistent and satisfactory results were given by the lead acetate method and it has been adopted for routine estimations.

The authors' thanks for permission to publish are due to Saml. Hanson & Son, Ltd., Eastcheap, London, in whose laboratories most of this work was carried out.

Reference

1. Kidd, J. D., Nanji, H. R., and Edwards, F. W., *Analyst*, 1941, **66**, 240.

37 Kelmscott Road
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A. Taylor
D. J. Taylor (Mrs.)
December, 1948

THE INACTIVATION OF BIOTIN BY CHLORINE

During a series of experiments in which the relative activities of D-biotin and DL-desthiobiotin to *Saccharomyces cerevisiae* were compared by a modification of the technique of Hertz,[1] it was observed that biotin gave variable low responses. The cause was traced to the occasional presence of small amounts of chlorine in the glass-distilled water used in one laboratory. Chlorine was found to inactivate aqueous solutions of crystalline D-biotin to different degrees, according to the chlorine concentration and time of contact. At the concentrations of biotin tested (10^{-6} to 10^{-9} M) and at constant time of contact t, $\log [B/(B - x)]$ was approximately proportional to chlorine concentration, where B = original biotin concentration and $(B - x)$ = biotin activity at time t. After 2 hr. contact at 0·01 parts per million, chlorine had no measurable effect on biotin activity, whereas at 3 parts per million, no detectable biotin activity remained. A concentration of 3 parts per million of chlorine did not affect the activity of aqueous solutions of DL-desthiobiotin. It may therefore be postulated that the sulphur atom of the biotin molecule is involved in a chemical reaction with chlorine and that the biotin is thereby inactivated.

The same concentration of chlorine did not affect biotin in the presence of other constituents of the assay medium, indicating that the chlorine was preferentially reduced by other oxidisable substances in the medium.

Since tap water may contain chlorine, which in certain types of stills is redissolved in the distilled water, it is important that water used for the dilution of biotin for microbiological assays should be tested for freedom from chlorine. If no visible colour is developed by the standard *o*-tolidine test,[2] the water is sufficiently free from chlorine to have no perceptible effect on the activity of biotin towards *S. cerevisiae*.

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RESEARCH AND DEVELOPMENT DEPARTMENT
DISTILLERS COMPANY LIMITED
EPSOM, SURREY

J. S. HARRISON
E. J. MILLER

January, 1949

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1949, **74**, 262).

Public Analyst	*Appointments*
HATFULL, Ronald Stanley (Deputy)	Metropolitan Borough of Battersea.
JAMES, George Vaughton (Temporary Deputy)	County Borough of Bournemouth.
LEATHER, Alfred Norman	County Borough of Manchester.
LYNE, Francis Arthur (Deputy) ..	Royal Borough of New Windsor.
„ „ „ (Joint) ..	City of Oxford.
McLACHLAN, Thomas	City of Oxford. Royal Borough of New Windsor. Urban District of Thurrock.
PAULLEY, William Minterne.. ..	Metropolitan Borough of Stepney.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

NOTIFICATION of the following appointment has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 262).

Official Agricultural Analyst	*Appointment*
LEATHER, Alfred Norman	County Borough of Manchester.

Ministry of Food

STATUTORY INSTRUMENTS

1949—No. 1303. The Meat Products and Canned Meat (Amendment No. 2) Order. 1949. Price 1d.

This Order, which came into force on July 12*th*, 1949,

(a) *provides that the use of meat fat in the manufacture of whale meat, vegetable, or fish products shall not make them meat products;*
(b) *prohibits the use of whale meat in meat products; and*
(c) *provides maximum prices for Mexican Meat and Gravy.*

—No. 1536. The Labelling of Food (Amendment No. 2) Order, 1949. Price 1d.

This Order, as from August 20*th*, 1949,

(a) *imposes special requirements as to the labelling of frozen peas; and*
(b) *transfers Christmas puddings from Table C in the First Schedule of the principal Order* (*S.R. & O.*, 1943, *No.* 1553) *to item* 8 *of Table A in that Schedule, thereby providing that notwithstanding the revocation of the Manufactured and Pre-packed Foods* (*Control*) *Order*, 1942 (see *Analyst*, 1949, **74**, 413), *the ingredients of Christmas puddings need not be specified in accordance with Article* 2 (3) *of the principal Order.*

—No. 1584. The Feeding Stuffs (Rationing) Order, 1949. Price 6d.

This Order, as from September 1*st*, 1949, *replaces the Feeding Stuffs* (*Rationing*) *Order*, 1943, *as amended.*

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specification, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, J. H. Lane, 7-8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee M/22—Cleanliness of Hessian for Upholstery.
CK(M)5607—Draft for Cleanliness of Used Hessian.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Estimation of Serum Vitamin A with Activated Glycerol Dichlorohydrin. A. E. Sobel and S. D. Snow (*J. Biol. Chem.*, 1947, **171**, 617–632)—The vitamin A contained in 1 ml. of serum can be estimated by measuring the colour formed with activated glycerol dichlorohydrin (G.D.H.). This reagent, unlike the Carr - Price reagent, is not sensitive to moisture, and so a simplified procedure can be used.

Reagents—N *Potassium hydroxide* in 90 per cent. ethanol, prepared from a stock solution of potassium hydroxide and absolute alcohol. *Activated G.D.H.*—Distil glycerol dichlorohydrin in presence of 1 per cent. by weight of antimony trichloride at a pressure of 10 to 40 mm. of mercury. Discard the first and last fractions. The distillate should be free from antimony trichloride, as shown by its remaining clear when water is added.

Procedure (with Coleman Spectrophotometer)—To 1 ml. of serum in a ⅝ × 4-in. test tube, add 1 ml. of 95 per cent. ethanol, and then mix the contents of the tube by tapping (or for saponification add 1 ml. of *N* potassium hydroxide in 90 per cent. ethanol to the serum, mix the contents, and then place the tube in an oven at 60° C. for 20 min.). Add 2 ml. of A.R. light petroleum (b.p. 30° to 60° C.), shake for 10 min. and then centrifuge for about 30 sec. Remove the solvent layer with a fine-tipped dropper and place in a ½ × 4-in. test tube. Extract with a further 2 ml. of light petroleum, shaking for only 5 min. (If the carotene is to be determined at 440 mμ., place the light petroleum in a cuvette graduated at 4 ml., make up to the mark, and read the absorption with filter PC–4. Transfer the light petroleum extract quantitatively to a ½ × 4-in. test tube.)

Evaporate the extract to dryness by placing the tube in a water-bath at 40° to 50° C. and passing a stream of nitrogen over the contents. Add 1 ml. of A.R. chloroform and then 4 ml. of G.D.H. Mix the solution with a flat-tipped stirring rod for 2 min., and then place it in a 50-mm. cuvette of capacity 2·8 ml. Measure the absorption with a 555-mμ. filter, against a blank of 4 ml. of G.D.H. and 1 ml. of chloroform. Turn the wavelength dial to 800 mμ. and, at 4 min. after the mixing of the reagents, measure the absorption, using filter PC–5.

Prepare a calibration curve for vitamin A, by means of standards in chloroform solution containing from 0·2 to 5·0 μg. per ml. Bring the vitamin A standard and the G.D.H. solution to 25° C. before use. Mix 1 ml. of standard and 4 ml. of G.D.H. solution in a glass-stoppered cylinder, and then place the latter in a water-bath at 25° C. for 2 min. Pour the solution into a 50-mm. cuvette and read the absorption with the 555-mμ. filter exactly as above. Plot a graph of optical density against the vitamin A concentration of the standard.

The calibration curve of carotene can be determined at 440 mμ. against a light petroleum blank, using standards containing 1 to 10 μg. of carotene per 4 ml. of light petroleum. The carotene calibration curve can also be determined with G.D.H. at 800 mμ., with standards in chloroform containing 1 to 10 μg. of carotene per ml. Proceed as for the vitamin A calibration, reading the absorption 4 min. after mixing the reagents.

Prepare a calibration curve as though for vitamin A with carotene at concentrations of from 1 to 10 μg. per ml. This graph is used to correct the sample readings for interference by carotene, the amount of carotene being determined at 555 mμ. or at 800 mμ.

The procedure is slightly modified when other photometers are used. With a Beckman spectrophotometer, determine the vitamin A at 555 mμ. and the carotene at 830 mμ. Dissolve the residue from the light petroleum extract in 0·2 ml. of chloroform and add 0·8 ml. of G.D.H. Measure the absorption in a 10-mm. cell of capacity 1 ml. The amount of serum required can be reduced by using smaller cells.

With visual colorimeters, dissolve the dried extract from 1 ml. of serum in 0·2 ml. of chloroform and then add only 0·8 ml. of G.D.H. Compare the colour of the sample solution and a standard in 1-ml. micro-cups of length 40 mm. Prepare a standard for each unknown with 1 ml. of a chloroform solution containing 2·5 μg. of vitamin A to which are added 4 ml. of G.D.H.

The G.D.H. method has been compared with the Carr - Price method, and good agreement found. Higher values were obtained with the Carr - Price reagent when saponified serum was used. Using the G.D.H. reagent, however, the same value was obtained on saponified and unsaponified serum, unless the vitamin A content of the serum was large.

Working in a humid atmosphere, the use of A.R. light petroleum without further purification, and the use of redistilled waste G.D.H. did not interfere with the determination. The carotene content could be estimated at 440 mμ. or, with sufficient accuracy, at 800 mμ. using the same solution as for the vitamin A determination. W. S. Wise

Determination of Amounts of Purine Nitrogen of the Order of 10 to 40 micrograms. Application to Nucleic Acids, Nucleoproteins, Tissues, and Micro-organisms. R. Vendrely (*Biochimica et Biophys. Acta*, 1947, **1**, 95–100)—A simple and rapid method is described for estimating small amounts of purine nitrogen of the order of 10 to 50 μg. The material to be analysed is hydrolysed in *N* hydrochloric acid. The purines liberated are separated in a pure state by two successive precipitations as a copper complex. The nitrogen in the purine bases is determined by the micro-Kjeldahl method.

HYDROLYSIS—Measure the liquid sample with a Linderstrøm-Lang pipette (or weigh tissues) into a small flask of total capacity 3 to 4 ml. and fitted with a ground-glass stopper. Add sufficient hydrochloric acid of a convenient concentration to give a final volume of 1·2 to 2·0 ml. of normal acid and connect the flask to a condenser consisting either of a long narrow glass tube, or, for hydrolyses of longer duration, a water-cooled condenser. Heating is best carried out in a paraffin-bath fitted with a thermostat. Immerse the micro-flasks to the rim in the liquid and heat for 2 hr., with stirring, for a nucleic acid, and 8 hr. for a complex substance. After hydrolysis, cool by immersion in cold water. Remove the hydrolysate with a teat-pipette and filter through a micro-filter (Jena 12 G3) directly into 5-ml. conical centrifuge tubes, where the precipitation of the purines is carried out. Filtration may be aided by slight suction, the centrifuge tube being placed inside a vacuum receiver. Rinse the small flasks two or three times with 0·5 ml. of water, which is then passed through the filter. The same filter can be used several times. The filtrate, 2 to 3 ml., contains all the purines. Make it alkaline to methyl red with 40 per cent. sodium hydroxide solution, stirring with a fine glass stirrer, then make acid again with several drops of 10 per cent. acetic acid solution.

PRECIPITATION OF THE PURINE—Place the tubes containing the hydrolysed sample in a small water-bath containing boiling water. If the heating at this stage causes the formation of a precipitate, remove it by centrifuging, transfer the liquid to fresh tubes by means of a teat-pipette, wash the precipitate with 0·5 ml. of water acidified with acetic acid, and add this wash water to the main fraction.

First precipitation—Add successively to the hydrolysate, with stirring, 5 drops of 30 per cent. pyrosulphite (metabisulphite, $Na_2S_2O_5$ or $K_2S_2O_5$) solution, and 4 drops of 10 per cent. copper sulphate solution. A fine precipitate forms, and gradually becomes coloured by liberation of copper oxide. Remove the stirrer and wash it with a few drops of water. Centrifuge for 5 min. at 3000 r.p.m. and collect the brownish precipitate, removing the supernatant liquid with a teat-pipette; 2 or 3 mm. of liquid should be left above the precipitate.

Decomposition of the copper complex—Take up the precipitate in 1·5 ml. of warm water and replace in the water-bath. Add 4 drops of neutral sodium sulphide solution made by saturating a 1 per cent. sodium hydroxide solution with hydrogen sulphide and adding an equal volume of 1 per cent. sodium hydroxide solution. Add 6 drops of 10 per cent. acetic acid solution, followed by 2 drops more if the liquid is slow in clearing, and stir. A more or less colloidal black precipitate of copper sulphide and sulphur is gradually formed by warming on the water-bath for 20 to 25 min. The liquid should be almost colourless and quite clear. Centrifuge and transfer the liquid, without loss, by means of a teat-pipette to a fresh series of conical tubes. Wash the pipette with about 0·5 ml. of water, which is then run on to the precipitate, repeat twice, again centrifuge, and add the supernatant liquid to the first one obtained.

Second precipitation—Again precipitate the combined liquids with pyrosulphite and copper sulphate as before, centrifuge for 5 min., remove the supernatant liquid and take up the precipitate in 3 or 4 successive small amounts of water (0·4 ml.) and transfer by pipette to special digestion tubes, 9 cm. long, 1 cm. in internal diameter, blown out to a bulb, 2 cm. across, on one side of the closed end.

DETERMINATION OF NITROGEN IN THE PRECIPITATED PURINE—*Digestion*—To prepare the digestion reagent, boil 50 ml. of pure sulphuric acid for 15 min. with an excess (5 g.) of a catalyst made by mixing 1 part of selenium with 5 parts of mercuric sulphate, and 30 parts of potassium sulphate. Decant the completely dehydrated acid, while boiling, into a pre-heated ground-glass stoppered tube, stopper, and allow to cool. Use the clear supernatant liquid as the reagent; it will keep in good condition for over a month if precautions are taken to prevent re-hydration.

Evaporate an aqueous suspension of purine complex to dryness in the digestion tube in an oven at 105° C. Drop into the tube several strands of glass wool that has previously been washed with chromic acid and water. Cut a small wick from very fine strands of the glass wool to a length of 3 to 5 mm. above the axis of the tube. Add 80 μl. of the digestion reagent, and place the tubes fan-wise on a 4-mm. thick asbestos plate perforated with small holes 2 mm. in diameter corresponding to the base of each tube, with the upper ends of the tubes resting on a metal frame. Heat the asbestos plate by a 600-watt electric element. The heating, for a total time of 30 min., is discontinuous, the current being so adjusted that the boiling liquid rises and falls up the side of the tube for a distance of about a third of the total length. When the digestion is complete, cool the tubes and add 1 ml. of water.

Distillation—Markhams' apparatus is used (*Biochem. J.*, 1942, **36**, 790). Transfer the liquid to the apparatus by means of a teat-pipette and wash out the digestion tubes twice with 1 ml. of water. Introduce 0·6 ml. of sodium hydroxide by slowly raising the stopper of the charging funnel, and rinse with an equal volume of water. Collect the distillate in 3·5-ml. receptacles, made by cutting off the neck of 5-ml. ampoules, and containing 90 μl. of $N/30$ sulphuric acid, 1 drop of 0·08 per cent. methyl red, and 4 drops of water. A narrow tube connected to the end of the condenser dips into the acid and at the conclusion of the distillation this tube is rinsed on the outside with 2 drops of water. Distillation of 2 ml. takes 5 to 7 min.

Titration—Use a 100-μg. Linderstrøm-Lang burette (*Compt. rend. Lab. Carlsberg*, 1931, **19**, No. 4). When determining the normality of the $N/30$ acid, choose a given colour for the end-point and use this colour in the following tests. At least 3 blanks and 3 tests should be run.

Calculation of results—A correction factor is applied according to the time of hydrolysis. For hydrolysis times of 0, 1, 2, 4, 8, and 16 hr. the values obtained for adenine are multiplied by 1·000, 1·010, 1·030, 1·045, 1·104, and 1·256, respectively. The corresponding correction factors for guanine are 1·000, 1·000, 1·010, 1·012, 1·036, and 1·075, and for an equimolecular mixture of adenine and guanine, 1·000, 1·012, 1·022, 1·038, 1·071, and 1·165. Reproducible results were obtained by this method on natural materials with an accuracy of about 5 per cent. J. S. Harrison

Determination of Phytic Acid by Oxidation of the Inositol with Periodic Acid. M. H. M. Heggen and J. F. Reith (*Pharm. Weekblad*, 1948, **83**, 801–805)—Phytic acid can be hydrolysed by boiling under refluxing conditions for 8 hr. with 35 per cent. sulphuric acid. The inositol can then be determined by oxidation with periodic acid under definite conditions, under which 6·73 atoms of oxygen are used for 1 molecule of inositol. The oxidation is carried out at 37° C. for 19 hr., after which sodium hydrogen carbonate is added, followed by excess of arsenite and potassium iodide. After 10 min., the excess of arsenite is determined by back titration. The presence of iron, as in precipitated iron phytate, does not interfere. G. Middleton

Gas Analysis

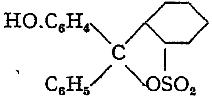
Rapid Determination of Low Concentrations of Carbon Monoxide in Air. M. Katz and J. Katzman (*Canadian J. Res.*, 1948, **26**, **F**, 318–330)—A granular form of 69 mol. per cent. of silver permanganate deposited on 31 mol. per cent. of zinc oxide oxidises carbon monoxide in air completely. The presence of some water vapour is necessary to initiate oxidation, but changes in humidity from 30 to 100 per cent. have no further effect on the reaction.

The granular reagent has an average particle size of 0·90 mm. and is prepared by uniform deposition of the silver salt on the zinc oxide carrier, pressing of the moist filter cake in a mould at 3 to 5 tons per sq. in., and air drying of the graded material at 60° C. for 72 hr. Dried samples stored in brown bottles retain their activity towards carbon monoxide during storage for 2 years at room temperature. Loss of oxygen from the silver salt during use eventually causes loss in activity. 20 to 25 Ml. of the reagent are sufficient for an 8-hr. continuous test on concentrations of carbon monoxide below 0·1 per cent. before the reagent loses its efficiency. The reagent was used in two vessels. One was a glass tube of 1-in. diameter with ten copper-constantan thermocouples embedded in the reagent at half-inch intervals. Concentrations of carbon monoxide of 183 p.p.m. were completely oxidised at rates of flow up to 12 litres per min. The other vessel contained 20 ml. of granules and fifty-five differential thermocouples. A straight line relationship between concentration of carbon monoxide (0 to 600 p.p.m.) and microvolts (0 to 11,000) was obtained at a rate of flow of 6·5 litres per min. Hydrogen is also oxidised but less effectively: no appreciable errors are introduced provided the concentration of hydrogen is not appreciably greater than that of the carbon monoxide. The method is readily applicable to the determination of carbon monoxide in the air in aircraft, motorised vehicles, mines, and buildings, for concentrations of 5 to 200 p.p.m. W. J. Gooderham

Organic

Acid-Base Reactions in Organic Solvents. Behaviour of Some Halogenated Derivatives of Phenolsulphonephthalein with Various Classes of Organic Bases in Benzene. M. M. Davis, P. J. Schuhmann, and M. E. Lovelace (*J. Res. Nat. Bur. Stand.*, 1948, **41**, 27–40)—The previous investigation of bromophthalein magenta (Davis and Schuhmann, *Ibid.*, 1947, **39**, 221; *Analyst*, 1948, **73**, 467) is extended to the sulphonephthaleins, and similar experimental methods are employed.

A number of the halogenated sulphonephthaleins have been isolated as colourless or nearly colourless solids and the colour of commercial specimens is believed to be due to impurities or to hydration. This colourless form is believed to possess the lactone (sultone) formula.



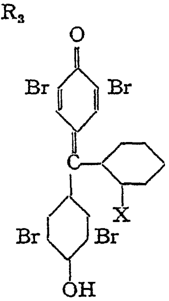
Solutions of these indicators in benzene are also colourless or nearly colourless, their structure being unaffected by such inert solvents. The pale yellow colour observed on standing is attributed to traces of moisture or to alkali in the glass surface. The yellow colour indicates conversion to one of the quinoid forms that are assumed in aqueous media. It disappears on addition of acetic acid. Transmittancy curves are plotted for benzene solutions of chlorophenol blue, bromophenol blue, iodophenol blue, tetrabromophenolphthalein, and bromophthalein magenta E down to 280 mμ.

The reactions of bromocresol green, bromophenol blue, and tetrabromophenol blue with various bases in benzene solution are illustrated by transmittancy curves. Qualitative observations are recorded for iodophenol blue, chlorophenol blue, bromochlorophenol blue, chlorophenol red, bromophenol red, bromocresol purple, and bromothymol blue. The following summary applies to all the above indicators with the exception of tetrabromophenol blue and bromothymol blue; chlorophenol red also shows slight variations.

Small quantities of either strong or weak bases cause the appearance or intensification of the yellow colour and this stage of the reaction is usually complete when approximately one equivalent of the base has been added. Further changes occur on addition of amines in excess of one equivalent and the final colour varies with the type of amine. Primary aliphatic amines cause precipitation, but a momentary red-purple colour is observed with some of the indicators. Secondary aliphatic amines produce a purple-blue colour. Tertiary aliphatic amines or *sym.*-di- or tri-arylguanidines produce a magenta colour. Quaternary ammonium salts give blue solutions. This second stage may require up to several hundred equivalents of amine for its completion. The unhalogenated sulphonephthaleins are not sufficiently soluble for similar investigation.

The transmittancy curve for the yellow form of bromophenol blue, produced by addition of one equivalent of 1 : 2-diphenylguanidine, is almost identical with the curve for bromophthalein magenta E with no added base. This is to be anticipated from the reasoned assumption that bromophenol blue, when treated with one equivalent of base, forms an acid salt of the structure represented by the following formula, where

$X = -SO_2\bar{O}\ H\overset{+}{N}R_3$



The same structure, with $X = -COOC_2H_5$, is the only structure that can be assigned to bromophthalein magenta E. The colour changes that accompany the further addition of various types of amines to solutions of the halogenated sulphonephthaleins in benzene are similar to those that occur with bromophthalein magenta E, and are attributed to the changes in configuration already postulated for bromophthalein magenta (Davis and Schuhmann, *loc. cit.*).

The usefulness of the sulphonephthaleins as indicators in hydrocarbon solvents is severely limited by their low solubility in such liquids and by the overlap in the steps in their neutralisation. The first change from the colourless to the yellow form is, however, brought about by aromatic, as well as by aliphatic amines, whilst bromophthalein magenta is not sufficiently acid to react with aromatic amines. W. C. JOHNSON

Piperazinium Salts for Utilisation in Identification of Organic Acids. M. Prigot and C. B. Pollard (*J. Amer. Chem. Soc.*, 1948, **70**, 2758–2759)—Thirty-six new piperazinium salts suitable for use in identifying organic acids have been prepared by an improved technique: they have been characterised and their properties are listed in the accompanying table.

DATA CONCERNING PIPERAZINIUM SALTS DERIVED FROM VARIOUS ORGANIC ACIDS

	Piperazinium salts			
			Neutral equivalents	
Acid	Yield per cent.	Melting-point ° C. corrected	Calcd.	Found
o-Benzoylbenzoic	78	186·2–186·6	287	289
o-Bromobenzoic	90	227–230 decomp.	244	241
m-Bromobenzoic	74	169–171	244	250
p-Bromobenzoic	49	224–226	244	240
α-Bromopropionic	73	195 decomp.	196	192
Dichloroacetic	62	181 decomp.	344	340
n-Capric	49	92·5–93·5	215	219
n-Caprylic	32	97·5–98·0	187	184
o-Chlorobenzoic	81	217–218 decomp.	200	194
p-Chlorobenzoic	67	219–220 decomp.	200	199
trans-Cinnamic	92	206 decomp.	191	190
Citric	100	141–142	139	137
Ethoxyacetic	73	120–121	147	145
p-Ethoxybenzoic	76	176·2–177·0 decomp.	209	211
Fumaric	98	240 decomp.	101	105
α-Furoic	94	234–236 decomp.	155	160
Gallic	84	209·0–209·7 decomp.	213	*a*
Hippuric	82	182–184 decomp.	222	222
Lauric	77	92·0–92·5	243	243
Maleic	92	148	101	102
Methoxyacetic	80	155·7–156·4	133	132
o-Methoxybenzoic	92	190·4–191·4	195	196
m-Methoxybenzoic	89	136·9–138·5 decomp.	195	202
α-Naphthoic	48	131·5–139·0 decomp.	215	216
β-Naphthoic	89	194·0–195·0 decomp.	215	217
p-Nitrocinnamic	90	247·9–248·7 decomp.	232	232
p-Nitrophenylacetic	96	205·5–205·9 decomp.	224	225
4-Nitrophthalic	96	201·5–204·5 decomp.	149	149
Pelargonic	60	95·1–96·2	201	198
Phenoxyacetic	84	183·7–184·2 decomp.	195	195
o-Phthalic	91	187–188	139	139
*iso*Phthalic	77	251·7–252·2 decomp.	126	126
Terephthalic	52	Decomp. above 350	126	127
D-Tartaric	86	248–254	118	117
meso-Tartaric	87	140–141	118	119
p-Toluic	79	203·0–203·3	179	181

a Solution too dark to titrate.

Preparation of derivatives—Dissolve some of the organic acid in anhydrous ether or *iso*propyl alcohol and gradually incorporate the calculated amount of piperazine, added as a 1 *M* solution in *iso*propyl alcohol. Separate the precipitate by filtration, add it to fresh ether or *iso*propyl alcohol, stir thoroughly, filter again, and dry in a desiccator over phosphorus pentoxide. If the sample is quite insoluble in ether or *iso*propyl alcohol, dissolve in water before adding piperazine, and evaporate over boiling water to recover the salts.

Determine the melting-point by using a bronze block pre-heated to within 5° of the m.p.

To determine the neutral equivalent, dissolve some of the salt in a 50 per cent. aqueous solution of *iso*propyl alcohol and titrate with 0·1 *N* sodium hydroxide, using either thymolphthalein or Orange II as indicator.

By the method employed the following acids failed to give salts of practical value for qualitative organic analysis: α-bromo-*n*-butyric, anthraquinone-β-sulphonic, barbituric, 2-chloropropionic, 2 : 5-dichlorobenzenesulphonic, 2 : 4-dichlorophenoxyacetic, diethylacetic, diphenylacetic, erucic, glycine, *p*-hydroxybenzoic, iodoacetic, itaconic, levulinic, DL-methylethylacetic, mucic, 1-naphthol-4-sulphonic, sulphosalicylic, thioglycolic, and trimesic.

A. H. A. Abbott

Determination of Aromatics and Olefines in Hydrocarbon Mixtures. C. Berg and F. D. Parker (*Anal. Chem.*, 1948, **20**, 456–457)—The determination is carried out by a modification of the acid-solubility method. The reagent used is 15 per cent. fuming sulphuric acid, which is added to a solution of the sample in glacial acetic acid. Experiments with fuming sulphuric and glacial acetic acids showed that addition of a mixture of these acids to the hydrocarbon resulted in only partial dissolution of aromatic compounds, whereas addition of fuming sulphuric acid to a solution of the hydrocarbon in glacial acetic acid was successful. Fuming sulphuric acid containing 15 per cent. of sulphur trioxide was more effective in dissolving olefines than acid of lower concentrations.

Solubility of hydrocarbons—*Iso*-octane (2 : 2 : 4-trimethylpentane) was used as one of the most sensitive of saturated hydrocarbons for solubility tests in four different mixtures of glacial acetic and fuming sulphuric acids. The solubility was only 0·5 per cent. compared with 3·0 per cent. for 98 per cent. sulphuric acid. Benzene, which is completely soluble in 98 per cent. sulphuric acid, was found to be soluble in a mixture of equal quantities of the two acids. The solubility of olefines was tested with di-*iso*butylene, diamylene (a mixture of 3 : 5 : 5-trimethylheptene-2 and 3 : 4 : 5 : 5-tetramethylhexene-2) and *iso*-octene (a mixture of isomeric octenes). The solubilities in the recommended mixed acids and in 98 per cent. sulphuric acid are given in Table I.

Procedure—Place 15 ml. of glacial acetic acid in a 60-ml. graduated bottle (Stoddard solvent test bottle, A.S.T.M. designation, D484–40) and add, by means of a pipette, 10 ml. of the sample followed by 25 ml. of fuming sulphuric acid (15 per cent. sulphur trioxide content) in 5-ml. portions. Shake the bottle in a bath of iced water after each addition and until no further heat is generated. Rotate the bottle for 15 min. at 30 r.p.m. Fill the bottle to the level with fuming sulphuric acid and allow to stand 2 hr. before reading the volume solubility. Add 0·5 per cent. to the upper meniscus reading to correct for the difference in curvature between the upper and lower menisci.

Results—The increased absorption given with this method compared with the 98 per cent. sulphuric acid method is particularly advantageous in the analysis of cracked gasolines. The latter method suggests a lower olefine content than is given by bromide - bromate determinations. Typical results are recorded in Table II.

Table I

Composition of sample, per cent. by volume							Solubility in	
n-Heptane	*iso*-Octane (*a*)	Di-*iso*-butylene	Di-amylene (*b*)	*iso*-Octene (*c*)	Toluene	Total aromatics and olefines %	Mixed acid	98% Sulphuric acid
100·0	—	—	—	—	—	0·0	0·0	0·0
—	—	100·0	—	—	—	100·0	80·0	42·0
—	—	—	100·0	—	—	100·0	93·0	45·0
—	—	—	—	100·0	—	100·0	99·0	49·5
—	—	—	—	—	100·0	100·0	100·0	100·0
50·0	—	30·0	—	—	20·0	50·0	50·0	32·0
50·0	—	—	30·0	—	20·0	50·0	50·0	31·0
50·0	—	—	—	30·0	20·0	50·0	50·0	33·5
50·0	—	10·0	—	—	40·0	50·0	50·0	45·0
50·0	—	—	10·0	—	40·0	50·0	50·0	45·0
50·0	—	—	—	10·0	40·0	50·0	50·0	50·0
20·0	20·0	10·0	10·0	10·0	30·0	60·0	60·0	48·5

Data for the solubility of certain pure hydrocarbons and the results of analyses of fuels are given.

The solubility method using 98 per cent. sulphuric acid is subject to error owing to the polymerisation of olefines and the formation of saturated hydrocarbons by complex reactions, *e.g.*, di-*iso*butylene and diamylene are particularly difficult to dissolve. Active saturated hydrocarbons are likely to dissolve giving rise to an error of the opposite kind that increases with increasing concentration of acid.

TABLE II

Gasoline	(bromide - bromate)	Solubility In 98% sulphuric acid	 In mixed acids
Straight-run gasoline from Los Angeles Basin crude oil	1·0	14·5	14·5
200° to 240° F. cut from thermally reformed Los Angeles Basin raffinate	21·4	22·0	29·0
Thermally reformed gasoline from Santa Maria Valley crude oil (0·44% sulphur)	32·0	28·0	37·0
Catalytic polymer gasoline (from C_3 –C_4 olefines)	76·0	38·5	74·5

W. C. WAKE

Determination of Asphaltenes, Oils, and Resins in Asphalt. R. L. Hubbard and K. E. Stanfield (*Anal. Chem.*, 1948, **20**, 460–465)—A method is described for determining three fractions of petroleum and native asphalts. These fractions are separated as asphaltenes, oils, and resins, and are recovered quantitatively. Since the separation of asphalts into these three components is necessarily arbitrary, it is essential, in order to obtain consistent and reproducible results, that definite chemical compounds rather than mixtures of specified boiling range should be used for solvent extractions, and that the extractions should be carried out at definite, reproducible temperatures. The authors use *n*-pentane and methanol-benzene for extractions at controlled temperatures in a special apparatus.

Apparatus—The two special pieces of apparatus used are shown in Figs. 1 and 2. Fig. 1 shows a glass adaptor for removing solvents and drying extracts at atmospheric or reduced pressures. It is used with 300-ml. flasks having flat bottoms and standard ground-joints, and flushing gas is passed through one side-tube on to the surface of the extract and out through the opposite tube. The constant temperature extraction apparatus shown in Fig. 2 allows the continuous extraction by liquid of solid or semi-solid materials in an inert atmosphere at specified temperatures. The water-bath D surrounding the solvent cooling-coil is maintained at the desired temperature by water circulating from a thermostatically controlled bath of larger capacity. The mercury-sealed paddle stirrer H is operated by an oscillating motor, G, of the type used for car windscreen wipers. The liquid trap K in the solvent return line aids observation of the colour of the extract. The sintered-disc crucible, shown in place in the extraction chamber, J, is of 50-ml. capacity with a disc diameter of

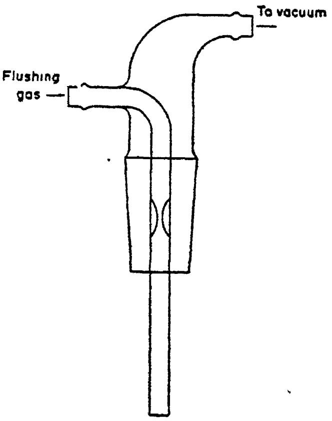


Fig. 1. Adaptor for removing solvent

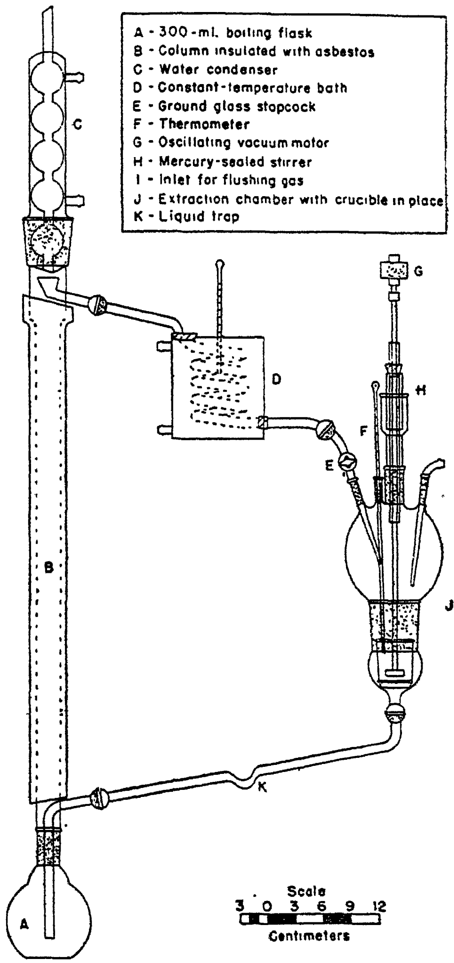

A - 300-ml. boiling flask
B - Column insulated with asbestos
C - Water condenser
D - Constant-temperature bath
E - Ground glass stopcock
F - Thermometer
G - Oscillating vacuum motor
H - Mercury-sealed stirrer
I - Inlet for flushing gas
J - Extraction chamber with crucible in place
K - Liquid trap
A
B
C
D
E
F
G
H
I
J
K
Scale
3 0 3 6 9 12
Centimeters

Fig. 2. Constant-temperature extraction apparatus

40 mm. and should be of such a porosity that 15 to 20 ml. of acetone will pass in 30 sec.

Preparation of asphalt sample—Weigh accurately into a 100-ml. centrifuge tube a sample containing 1·25 to 1·5 g. of asphalt, the lower weight being taken when the asphalt is known to contain a high proportion of resins or asphaltenes. Warm the tube to soften the asphalt and distribute it evenly over the lower part of the tube.

Determination of asphaltenes—Add to the sample in the centrifuge tube 40 ml. of *n*-pentane per g. of asphalt. Disperse by hand with a screw-type stirring rod made by flattening and then twisting a section 5 cm. long and situate 1 cm. from the lower end of a glass rod 6 mm. in diameter and 25 cm. long. Transfer the tube to a water-bath at 15·6° C. and, with the same rod, stir at 2500 r.p.m. for 10 min. Remove the stirring rod, place a stopper in the tube, and allow to stand for 12 hr. or overnight in darkness or subdued light. Return the tube to the constant temperature bath for 20 min., stir at 2500 r.p.m. for 10 min., and then remove it from the bath and centrifuge for 5 min. with a relative force of 975 *g*. Decant the clear pentane solution into a 300-ml. boiling flask and note the approximate volume of the pentane-insoluble fraction. Add to the residue 25 ml. of *n*-pentane per ml. of residue, stir for 10 min. at 15·6° C., and centrifuge for 5 min. as before. Repeat this washing process three times more, using the same volume of *n*-pentane each time. Add the washing liquid to the original pentane extract in the 300-ml. flask.

Dissolve the pentane-insoluble asphaltene fraction in benzene and filter the solution through filter paper into a tared boiling-flask. Use a few drops of methanol in eluting the final traces of asphaltenes from the filter paper. Connect the adaptor shown in Fig. 1 to the flask and distil to dryness on a steam-bath, a current of carbon dioxide, nitrogen, or helium being passed through the adaptor during the operation at a reduced pressure of 5 to 10 in. of water. After removal of the solvent, complete the drying of the extract for 25 min. at 105° C., all the time passing a stream of the flushing gas at reduced pressure. When dry, cool in a vacuum desiccator, flush with air, remove the adaptor, and weigh to determine the yield of asphaltene fraction.

Determination of oils—Distil the combined pentane extract and washings from the initial extraction to remove most of the solvent. If this extract is allowed to stand before further treatment, the air in the flask should be replaced by an inert gas. Evaporate this concentrated extract to approximately 5 ml. and pour it evenly over 25 g. of alumina in a Gooch crucible. (The alumina, of technical grade, should pass a 100-mesh per inch sieve and be retained on 200-mesh sieve. Heat the alumina for 2 hr. at 700° C. and cool in a desiccator before use.) Rinse the material on to the alumina with 3- to 5-ml. portions of *n*-pentane, continuing the rinsing until the base of the crucible becomes moistened with solvent. When correctly done the upper portion of the alumina is dark and the lower portion colourless. Place the crucible in the extraction chamber of the apparatus shown in Fig. 2 and dry the material to a powder by stirring gently in a stream of inert gas. Place *n*-pentane that has been dried by passing through alumina, in the boiling flask A, bubble gas slowly through the liquid, and heat the latter with a water-bath. Adjust the stopcock E so that *n*-pentane, cooled to 15·6° C. by the cooling coil, is added slowly to the sample in the crucible, the flow being such as to maintain it one-half to three-quarters full. The initial percolate from the crucible should be colourless or slightly yellow. If it is distinctly coloured, repeat the analysis with a smaller sample. After percolation has commenced, start the stirrer, and continue the extraction for 1 hr. Then wash the extraction chamber and the return line with *n*-pentane, remove the boiling flask, and concentrate the extract and washings to 25 ml. by distillation. Filter through filter paper, dry, and weigh as described for the asphaltene fraction.

Determination of resins—Remove the crucible containing the mixture of resins and alumina from the extraction chamber and mount on a filter funnel. Elute resins by alternately stirring with 10- to 20-ml. portions of a methanol - benzene mixture (10 + 90) and withdrawing liquid by suction. Distil the resin extract to a small volume, centrifuge to remove fine particles of alumina, filter into a tared boiling flask, dry, and weigh as before.

The determination of all three fractions can be completed in one 8-hr. day after the sample has been dispersed in *n*-pentane and allowed to stand overnight.

Results—Table I, based on 41 analyses of 16 asphalts, indicates the accuracy of the method. The deviations refer to those from the mean for each asphalt and are given in grams per 100 g. of sample.

Comparison of the new method with that of Strieter (*J. Res. Nat. Bur. Standards*, 1941, **26**, 415–418) is given for 6 coating asphalts. The average yields of asphaltene, oil, and resin fractions by the present method (referred to as the Bureau of Mines method) were 0·6 per cent. higher, 1·9 per cent. lower, and 1·2 per cent. higher, respectively, than those of the corresponding fractions determined by the Strieter method.

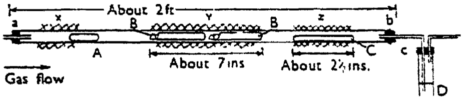
Table I

	Asphaltenes	Oils	Resins
Mean deviation	0·19	0·40	0·43
Maximum deviation	0·6	1·3	1·2
Percentage of samples with deviations greater than 0·50%	2	20	32

W. C. Wake

Estimation of Chlorine in Polymeric Materials. W. M. Phillips (*Plastics*, 1948, **12**, 587–589)—The method described is a simple combustion not requiring the use of special apparatus. Results are quoted for polychloroprene, polyvinylchloride, and a copolymer of the latter with vinylidene chloride.

Apparatus—The apparatus is shown in the diagram and consists of a hard glass tube of internal diameter ⅜ in. covered with rolls of wire gauze at X, Y, and Z. Oxygen is passed into the tube from the end a and the sample is contained in the platinum boat A placed at a short distance from Z-sectioned platinum contacts B, which are heated with a long burner over the length of the wire gauze. The long, porcelain boat, C, contains halogen-free barium carbonate and is heated by a burner. Rubber bungs are used for the connections at a, b, and c. A guard bubbler containing acidified silver nitrate solution is provided at D. Should the solution in this become turbid during combustion the combustion must be abandoned since it shows that the hydrochloric acid formed has not all been absorbed by the barium carbonate.



Procedure—Support the combustion tube at both ends by retort stands, insert the platinum contacts after re-etching in warm dilute *aqua regia*, fill the long boat, C, level with halogen-free barium carbonate and insert through the end b. Pass oxygen at 11 to 12 ml. per min., and heat the tube (surrounded by wire gauze) to about 600° C. at positions Y and Z. While the tube is heating, weigh out about 20 mg. of sample and then disconnect momentarily at a and insert the sample. Burn slowly so that complete combustion takes about 1 hr. After combustion is complete cool the tube in a stream of oxygen. When cool, withdraw the boat of barium carbonate and suspend its contents in water in a porcelain dish. Titrate the barium chloride formed with 0·025 *N* silver nitrate in the presence of a few millilitres of starch solution and dichlorofluorescein indicator.

Results obtained—Re-crystallised o-chlorobenzoic acid was used as a standard reference compound. The results obtained with polymeric materials are compared in the table with those expected and with some obtained by the Carius method. A micro-balance was used in earlier work, but later results were obtained with an aperiodic balance of the usual sensitivity.

		Chlorine, per cent.			
Polymer	Weight of sample	Individual	Mean	Expected	Carius method
Polychloroprene	20 mg.	40·34; 40·33	40·34	40·05	—
Polyvinylchloride	20 mg.	55·98; 55·65; 55·61; 55·63	55·64	56·73	55·9
Vinylchloride-vinylidenechloride copolymer	15 mg.	65·42; 65·30; 65·71; 66·12	65·64	64·93	65·3

W. C. Wake

Carborundum Boiling Stones in Micro-Kjeldahl and Other Digestion Procedures. D. Fraser and R. S. Baker (*Anal. Chem.*, 1948, **20**, 1124)—Carborundum has the advantages that it is non-porous, indestructible, and heavy enough to settle readily even when finely-divided, and it is quantitatively removed in decanting the supernatant liquid. No nitrogen-error is incurred by its use and digestion is more efficient than with glass beads. Twenty- to 50-mesh particles are most satisfactory; they should be washed several times by boiling with distilled water, and oven-dried before storing ready for use. M. E. Dalziel

Selenium as Catalyst in Kjeldahl Digestions. S. M. Patel and A. Sreenivasan (*Anal. Chem.*, 1948, **20**, 63–65)—A summary of the published observations on the use of selenium as a catalyst in Kjeldahl digestions is given, and the results of a study of the effect of time of digestion on recovery of nitrogen are presented. The standard apparatus and procedure were used. Samples containing 10 mg. of nitrogen were treated with 20 ml. of concentrated sulphuric acid, 6 g. of potassium sulphate, and 0·2 g. of crystalline copper sulphate. As catalyst, 50 mg. of selenium were added and, in some experiments, 0·5 g. of mercuric oxide was added in addition to the selenium. Mercury-ammonium complexes were destroyed by adding 0·5 g. of sodium thiosulphate before distillation.

With ammonium sulphate and using selenium, the recovery of nitrogen was 99·7 per cent. after 10 min. digestion and was reduced to less than 95 per cent. by 3 hr. digestion. With mercuric oxide and selenium present, recovery was complete after 20 min. digestion, and 98·6 per cent. after 3 hr., the slower rate of loss of nitrogen being due to the formation of mercuric-ammonia complexes. No loss of nitrogen occurred when neither of the catalysts was added. In the digestion of casein in absence of selenium, the liquid became clear after 40 min.; recovery of nitrogen was complete only after another hour's digestion and was slightly low if the digestion was further prolonged. In presence of selenium, the liquid was clear after 10 min., recovery was complete after another 15 min., and fell to 95·6 per cent. when the time of digestion after the liquid cleared was 6 hr. When mercuric oxide was present the effect of over-long digestion was not so great. Loss of nitrogen caused by the selenium takes place only when conversion of nitrogen to ammonium sulphate is complete.

Recovery of nitrogen from nicotinic acid and from quinoline was not complete when the time of digestion after the liquid became clear was 6 hr. Recovery of nitrogen increased with digestion time, but after 6 hr. the liquids began to bump to an undesirable extent and the digestion could not be continued. Only when both selenium and mercuric oxide were present was the recovery of nitrogen over 98 per cent. after 6 hr. digestion.

The time of after-boiling required for obtaining theoretical yields of nitrogen must be standardised for each type of nitrogenous organic matter, and selenium cannot be recommended as a general reagent for Kjeldahl determinations.

B. Atkinson

Detection and Estimation of Microquantities of Cyanide. A. O. Gettler and L. Goldbaum (*Anal. Chem.*, 1947, **19**, 270–271)—The sensitivity of the Prussian blue test, which is specific for cyanide, is enhanced by conducting hydrogen cyanide through a piece of filter paper impregnated with ferrous sulphate and sodium hydroxide.

Method—The exit tube from the reaction vessel is in two parts, the adjacent ends of which are flanged, ground, and lugged, to enable a disc of test paper to be held across the tube firmly when the lugs are held together by rubber bands. The paper is prepared by dipping a sheet of Whatman No. 50 smooth glazed, acid- and alkali-treated paper for 5 min. in a filtered, 10 per cent. solution of hydrated ferrous sulphate, drying it in air, dipping it in 20 per cent. sodium hydroxide solution, and again drying in air.

Procedure—Place 2 ml. of test solution or 2 g. of finely-macerated tissue in a 50-ml. aeration tube with 3 ml. of water. Acidify with dilute sulphuric acid, or with 20 per cent. trichloroacetic acid solution for blood samples. Connect one end of the assembled exit tube to the aeration tube and the other end to an aspirator. Place the aeration tube in water at 90° C., submerged so that the liquid levels are the same, and apply suction at the maximum rate for 5 min. Remove the test paper and place it in diluted hydrochloric acid (1 + 4) to remove iron hydroxides, rinse with water, and dry. The intensity of the blue stain is proportional to the cyanide concentration; the limit of sensitivity is 0·1 μg. of hydrogen cyanide.

The quantity of cyanide is estimated by comparison with standard stains. For best results, the diameter of the flange orifice should be adjusted according to the amount of cyanide, *i.e.*, 4-mm. diameter for 0·2 to 1 μg., 10-mm. diameter for 1 to 5 μg., and 15-mm. diameter for 5 to 20 μg. Results are correct to within 0·1 μg. on quantities up to 1 μg., and to within 1 μg. on quantities between 1 and 5 μg.

To detect nitrogen in organic compounds, embed a small crystal of the compound in a small piece of metallic sodium and fuse in a piece of Pyrex tubing sealed at one end. Plunge, while red hot, into 2 ml. of water and treat the solution as above.

M. E. Dalziel

Inorganic

Determination of Hydrogen Chloride in Presence of Chlorine. H. N. Barnam and T. R. Thomson (*Anal. Chem.*, 1948, **20**, 60–61)—From an aqueous solution containing hydrochloric acid and chlorine the chlorine is removed by boiling the solution under refluxing conditions and the hydrochloric acid is then titrated with standard alkali. With the ordinary type of reflux condenser removal of chlorine is slow, but with the apparatus illustrated removal of chlorine is complete after boiling for 15 min. Analyses of 0 to 1 *N* solutions of hydrochloric acid containing dissolved chlorine showed that all the results for normalities above 0·008 were high by about 0·0007 and the error was higher for solutions containing less acid. Standard hydrochloric acid should therefore be added to raise the normality above 0·1 before the chlorine is boiled out.

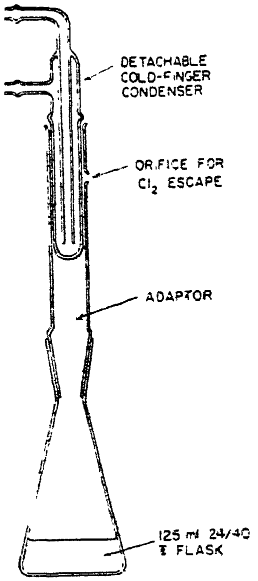


Fig. 1. Recommended boiling apparatus

B. Atkinson

Electrometric Titration of Nitric Acid in Oleum. C. D. McKinney (jun.), W. H. Rogers, and W. H. McNabb (*Anal. Chem.*, 1947, **19**, 1041)—An electrometric method is described for the determination of the amount of nitric acid added to oleum as an anti-freeze. Bowman and Scott (*Ind. Eng. Chem.*, 1915, **7**, 766) recommend titration of the nitric acid with a standard solution of ferrous sulphate, in which the first appearance of the red-brown colour of the ferrous nitrosyl sulphate is taken as the end-point of the titration. The end-point is easily obscured if the oleum is dark in colour, as in the reclaimed sulphuric acid used as drip acid for absorption of sulphur trioxide in the manufacture of oleum.

A previous report by Treadwell and Vontobel (*Helv. Chim. Acta*, 1937, **20**, 573) on the electrometric titration of nitric acid required the use of a potentiometer and a specially prepared electrode and titration cell. In the present work the Serfass electron-ray titration apparatus (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 536) fitted with a platinum - tungsten electrode combination was used. This has the advantage of convenience, elimination of interference of the dark colour with the end-point, and elimination of the empirical blank correction of about 0·2 ml. Any similar assembly should serve equally well.

Procedure—To prepare the ferrous sulphate solution (approximately 0·6 *N*) dissolve 176·5 g. of ferrous sulphate heptahydrate in about 400 ml. of water, add 500 ml. of 60 per cent. sulphuric acid, dilute to 1 litre, and standardise the solution against 0·5 *N* potassium dichromate in presence of 10 ml. of 18 *N* sulphuric acid in the manner described (*infra*) for determination of nitric acid.

Weigh a sample of oleum containing about 0·4 g. of nitric acid from a Lunge pipette into a 250-ml. beaker containing 100 ml. of concentrated (95 per cent.) nitric-free sulphuric acid. Turn the sensitivity control of the titration unit to position eight and set the polarisation control in the off position. Place the electrodes and the mechanical stirrer in the beaker and mix the solution thoroughly. Adjust the cathode-ray tube to the closed position by means of the "eye control." Titrate with the ferrous sulphate solution; addition of the first few millilitres will cause the "eye" to open momentarily but further slow addition will bring it back to the closed position. Continue the titration until the eye remains open for 30 sec.

Determine the nitrosyl sulphuric acid by titration with standard potassium permanganate. Weigh a 10-g. sample of oleum into a 100-ml. volumetric flask containing about 20 ml. of 95 per cent. sulphuric acid, dilute to the mark with sulphuric acid, and then transfer an aliquot (25 ml.) to a 250-ml. beaker containing water and a large piece of ice. Titrate the solution with standard potassium permanganate to an end-point persisting for 1 min.

When the procedure was applied to two samples of oleum that had been analysed by the familiar nitrometer method, it was noted that the electrometric end-point preceded the visual end-point by 0·12 ml., thereby indicating that this excess of ferrous sulphate solution is needed to produce a detectable colour change. No correction is necessary in calculating the results of the electrometric method.

The ferrous sulphate titration of nitric acid in oleum measures only the actual nitric acid content of the sample, whereas the nitrometer method measures the nitrosyl sulphuric acid also. The nitrosyl sulphuric acid occurs owing to reaction between oxides of nitrogen and oleum. To place the two methods on the same basis therefore, it was necessary to determine the nitrosyl sulphuric acid content of the oleum samples and to correct the results of the ferrous sulphate titration.

Concordant results were obtained by this method over a wide range of sample size and the values obtained agreed well with those of the nitrometer method. Although the method has been applied only to oleum in this investigation, it can be applied equally well to inorganic and organic nitrates.

A. O. Jones

Rapid Oxidimetric Procedure for Determining Nitrate. W. Leithe (*Mikrochem.*, 1947, **33**, 48–50)—Ferrous sulphate is oxidised to the ferric state by nitric acid on boiling in a solution containing about 65 per cent. of sulphuric acid by weight. The excess of ferrous iron is determined with potassium dichromate or permanganate.

For large amounts of nitrate—Dissolve 1 g. of the nitrate-containing material in water and dilute accurately in a 500-ml. volumetric flask. Pipette 25 ml. into a 250-ml. Erlenmeyer flask. Pipette 25 ml. of ferrous sulphate solution (55 g. of the heptahydrate dissolved in 100 ml. of water containing a few drops of sulphuric acid, and diluted to 1 litre with 50 weight-per cent. sulphuric acid of sp.gr. 1·40) into the flask, followed by 25 ml. of concentrated sulphuric acid, and 3 small boiling beads, and heat to boiling for 3 min. The dark brown solution becomes light yellow. Cool the solution in water, add 3 to 5 ml. of 70 to 80 per cent. phosphoric acid solution and 50 ml. of water, and cool again. Add 2 drops of 0·025 *M* ferroin solution and titrate with 0·1 *N* potassium dichromate to the appearance of a blue-green colour, or titrate with 0·1 *N* potassium permanganate without an indicator. Carry out a blank determination.

For small quantities, 0·03 *to* 3 *mg.*, *of nitrate ion*—Into a 100-ml. Erlenmeyer flask pipette 5 ml. of 0·02 *N* ferrous sulphate (5·5 g. of heptahydrate dissolved in 20 ml. of water and diluted to 1 litre with 50 per cent. sulphuric acid) and 5 ml. of concentrated sulphuric acid. Remove the air by adding about 0·2 g. of sodium or potassium hydrogen carbonate and add the sample dissolved in 5 ml. of water. Mix the solution and boil it gently for 3 min. in presence of beads to prevent bumping; then add 1 ml. of phosphoric acid and 5 ml. of water and cool the solution. Titrate with 0·01 *N* potassium dichromate, using 0·0025 *M* ferroin as indicator.

Ammonium salts, chloride, and ferric iron do not interfere with the method, but nitrite interferes with the back-titration.

M. E. Dalziel

Accumulation of Traces of Arsenate by Co-precipitation with Magnesium Ammonium Phosphate. [Determination of Small Amounts of Arsenic in Steel.] I. M. Kolthoff and C. W. Carr (*Anal. Chem.*, 1948, **20**, 728–730)—A systematic study to determine the best conditions for the quantitative co-precipitation of arsenate has been made.

Procedure—Add to the arsenic solution sufficient bromine water to oxidise all the arsenic present and produce a yellow colour. Add sufficient monopotassium phosphate to provide 1 mg. of P_2O_5 per ml. of solution, add 1 ml. of hydrochloric acid, and 10 ml. of magnesia mixture (50 g. of magnesium chloride hexahydrate and 100 g. of water). Neutralise with aqueous ammonia solution and, when most of the precipitate has formed, add 5 ml. of concentrated aqueous ammonia solution in excess. Filter the solution after 2 to 4 hr., wash the precipitate with diluted aqueous ammonia solution (1 + 19) and determine the arsenate in the precipitate by a suitable volumetric procedure

(*Volumetric Analysis*, I. M. Kolthoff and N. H. Furman, Vol. 2, p. 410, 1929).

A blank determination should be made as a check on the reagents.

In 500 ml. of solution, 0·075 mg. of arsenic can be determined to within 2 per cent.

Determination of arsenic in steel—Treat a sample containing not less than 0·1 mg. of arsenic in a 250-ml. Erlenmeyer flask with 25 ml. of 6 *N* nitric acid and a small excess of bromine water, boil for a few minutes, and cool the solution. Add 10 g. of tartaric acid for each gram of steel taken, 0·2 g. of potassium dihydrogen phosphate, and 50 ml. (a ten-fold excess) of magnesia mixture. Transfer to a 250-ml. bottle and add, at least, a 10-ml. excess of concentrated aqueous ammonia solution. Shake for 4 hr., filter off the precipitate, and dissolve it in 3 *N* hydrochloric acid. Add 1 g. of tartaric acid, 10 ml. of magnesia mixture, and an excess of aqueous ammonia solution. Shake the vessel for 2 hr., filter, and wash the precipitate with diluted aqueous ammonia solution (1 + 19). Re-dissolve the precipitate in 3 *N* hydrochloric acid and determine the arsenate as usual.

For amounts of the order of 0·1 mg. of arsenic, use a micro-burette and 0·005 *N* iodine..

On a sample containing 0·012 per cent. of arsenic, the eight values obtained ranged from 0·011 to 0·014 per cent. On samples containing less than 0·01 per cent. of arsenic, the blank corrections are too large for the method to be satisfactory.

A similar method can be used for determining small amounts of arsenic in the presence of antimony, tin, aluminium, or zinc, the precipitation of these metals by ammonia being prevented by adding tartaric acid; with antimony, a re-precipitation may be necessary. M. E. Dalziel

Preparation of Phosphomolybdic Acid from Phosphoric Acid and Molybdic Trioxide. T. J. Hastings, jun. and H. A. Frediani (*Anal. Chem.*, 1948, **20**, 382–383)—The method of preparation suggested by Linz (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 459) uses only water as solvent and is convenient for preparing commercial quantities of the acid. Reaction between 1 g.-mol. of molybdic trioxide and 0·083, 0·1, or 0·166 g.-mol. of phosphoric acid, filtration, and crystallisation gave initial products containing phosphorus : molybdenum ratios 1 : 10·11, 1 : 8·74, and 1 : 6·15 respectively, but on extraction of their aqueous solutions with ether, addition of water and a small amount of nitric acid, and crystallisation all gave good yields of an acid containing one phosphorus to 11·93 ± 0·08 molybdenum atoms.

When amounts of tungstic trioxide and phosphoric acid calculated to yield the 1 : 12 acid were treated by the Linz method no 1 : 12 acid was obtained; a ten-fold excess of phosphoric acid also failed to give this acid. Freshly-precipitated tungstic acid was not tried. M. E. Dalziel

Iodine Monochloride End-point in Titration of Tripositive Antimony. Titration with Iodate, Permanganate, and Ceric Solutions. E. W. Hammock, R. A. Brown, and E. H. Swift (*Anal. Chem.*, 1948, **20**, 1048–1050)—Accurate results are obtained only in a narrow range of hydrochloric acid concentrations.

Iodate solutions—At acid concentrations below 2 *M*, iodate and iodine monochloride are fairly rapidly reduced by tervalent antimony to iodine, which is only slowly oxidised to iodine monochloride by iodate. Above 4 *M*, reduction is slow, and at 5 *M*, it is incomplete. A concentration between 2·5 and 3·5 *M* is recommended; increase of either hydrogen ion or chloride ion concentration results in a decreased rate of oxidation of the antimony.

Permanganate solutions—When the iodine monochloride end-point is used, the concentration of hydrochloric acid must be not greater than 3 *M* indicating that permanganate has a greater tendency than iodate to oxidise iodine selectively at high acid concentrations. Moderate variations in iodine monochloride and antimony concentrations do not cause marked changes in titration characteristics.

Ceric sulphate solutions—Precipitates are liable to be formed in solutions less acid than 2 *M*. Below 3 *M*, the tendency is to over-run the end-point because of the slow oxidation of the iodine. Above 3 *M*, the slow oxidation of the antimony tends to cause premature end-points. Thus the iodine monochloride end-point is not recommended with ceric solutions. In absence of iodine monochloride, antimony is more quickly oxidised by ceric ions in 6 *M* than in 2 *M* hydrochloric acid, so it appears that iodine is preferentially oxidised by ceric ions and the slow oxidation of antimony by iodine monochloride is the limiting factor.

Titrations were carried out (1) with permanganate, essentially according to McNabb and Wagner (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 251), but in 2 *M* acid and below 5° C.; (2) with ceric sulphate in 3 to 3·5 *M* acid, using methyl orange as indicator; (3) with iodate in 2·5 to 3 *M* acid; and (4) with permanganate, using the iodine monochloride end-point in 2·5 to 3·5 *M* acid. All values agreed to within 1 part in 1000. M. E. Dalziel

Determination of the Gases in Meteoritic and Terrestial Irons and Steels. L. K. Nash and G. P. Baxter (*J. Amer. Chem. Soc.*, 1947, **69**, 2534–2544)—The methods available for the determination of gases in steels and meteorites are reviewed. Thermal methods of extraction, such as fusion *in vacuo*, are considered to be inadmissable owing to reactions at high temperatures between constituents of the evolved gases and between the gases and the sample. Dissolution of the sample in aqueous solutions of salts overcomes this difficulty and tests have been carried out to investigate the relative merits of solutions of potassium cupric chloride, iodine, and mercuric chloride for this purpose.

Gas-free steels prepared by fusion *in vacuo* were used to determine the blanks caused by the reagents, and the recovery of synthetic gas mixtures maintained in contact with the reagent solutions was also studied. The use of hot solutions was found always to be undesirable, the evolution of gases from side-reactions being considerable. Subsequent tests were carried out with cold solutions, using mechanical shaking to increase the rate of dissolution of the sample.

Potassium cupric chloride—Considerable amounts of carbon dioxide were obtained in blank tests. The recovery of carbon monoxide was incomplete owing to retention by cuprous salts; the recovery of nitrogen and hydrogen was satisfactory.

Iodine—The amounts of carbon monoxide and carbon dioxide evolved in blank tests were very much greater than for the other two reagents.

Mercuric chloride—The recovery of synthetic gas mixtures was satisfactory. The carbon monoxide and carbon dioxide blanks were small except with steels of high carbide contents. The evolution of blank gases continued after dissolution of the sample was complete, and this secondary evolution was related to the primary evolution of blank gases during dissolution of the sample. Hence by analysis of the gases evolved during the secondary evolution, a correction could be deduced for the gases evolved from side-reactions during a determination.

Mercuric chloride is the most satisfactory reagent, but potassium cupric chloride may be used if results for nitrogen and hydrogen only are required. The following general procedure is recommended.

Procedure—The reaction is carried out in a flask fitted with a stop-cock and a short condenser, and having a sealed side-arm to contain the sample. Load the sample into the side-arm, and transfer the reagent solution to the flask, using a volume of about 250 ml. containing sufficient reagent to give a 20 per cent. excess. Boil the solution gently under slight suction to remove air, close the stop-cock, and freeze the reagent solution to minimise the vigour of the initial reaction. Tip in the sample, remove the flask from the freezing bath, and shake it mechanically until dissolution is complete. Draw off and analyse the evolved gas. If mercuric chloride solution has been used, continue the shaking, and apply a correction based on the composition of the secondary evolution.

H. J. Cluley

Identification of Free Silica in Dusts and Fumes. W. H. Gitzen (*Anal. Chem.*, 1948, **20**, 265–267)—Free silica can be detected in dusts or fumes by observing in the electron microscope the effect of exposing samples to hydrofluoric acid vapour. The method has been used for detecting silica in fumes occurring in the production of fused alumina from siliceous materials.

The dust sample is prepared for examination by dispersion in a resin solution (*e.g.*, Formvar), which is spread into a film by pouring it on to glass or water. Alternatively, the sample is prepared during collection by lining a small electrostatic precipitator with a nitrocellulose film supported on a screen.

After preliminary examination under the electron microscope, the sample film is exposed for 1 to 5 min. to the vapour from 48 per cent. hydrofluoric acid solution at room temperature. The fine particles of silica are completely vaporised as silicon tetrafluoride and leave in the film voids that are readily observed on re-examination under the electron microscope. Silicates are indicated by voids containing some unvaporised material. Alumina is unaffected by the treatment.

H. J. Cluley

Inorganic Chromatography. L. Sacconi (*Gazz. Chim. Ital.*, 1948, **78**, 583–591)—The hypothesis has been advanced that the adsorption of metallic ions on a column of alumina is determined by the alkalinity of the alumina.

To test this, trials have been made with two types of alumina, the first being technical alumina activated by heating to 600° C. for 10 hr., and the second being prepared by oxidation of amalgamated aluminium cuttings in moist air and subsequent heating of the oxide thus formed to 650° C. for 10 hr. Thus prepared, the alumina contained no trace of sodium and its suspension in water had pH 5·4. An aqueous suspension of technical alumina has pH about 9·4.

Columns prepared with these two types of alumina were tested with solutions of mercuric nitrate and of thallic chloride. The coloured zones obtained, yellow for mercury and brown for thallium, were similar with each alumina specimen.

Suspensions of alumina were made by shaking with water in test tubes. These were allowed to settle and a solution of mercuric chloride was added cautiously. In the tube containing technical alumina, a pale yellow precipitate was formed in the clear liquid and, on shaking and allowing to settle again, the alumina under the stratum of yellow assumed a reddish colour. With the alumina from amalgamated metal no precipitate was formed in the clear liquid but, on shaking, the alumina assumed a colour varying from ochre to reddish. Similar results to these latter ones were obtained with technical alumina that had been subjected to prolonged washing with water.

It is concluded therefore that whilst the adsorption of cations is due to the formation of basic compounds this is caused not by the alkalinity of the liquid but by a reaction at the surface of the alumina itself.

Experimental—The columns employed were 3 mm. in diameter and 10 cm. long, and were filled with technical alumina activated as described and sifted through sieves of 625 to 1600 apertures per square centimetre. The cations separated in the following order:—

$$\begin{array}{lllllll} & & & \mathrm{Ti}^{\cdot\cdot\cdot\cdot} & & & \\ & & & \mathrm{U}^{\cdot\cdot\cdot\cdot} & & & \\ \mathrm{As}^{\cdot\cdot\cdot}, & \mathrm{Sb}^{\cdot\cdot\cdot}, & \mathrm{Bi}^{\cdot\cdot\cdot}, & \mathrm{Cr}^{\cdot\cdot\cdot}, & \mathrm{Hg}^{\cdot\cdot}, & \mathrm{UO_2}^{\cdot\cdot}, & \mathrm{Pb}^{\cdot\cdot}, \\ & & & \mathrm{Fe}^{\cdot\cdot\cdot} & & & \\ & & & \mathrm{Tl}^{\cdot\cdot\cdot} & & & \\ & & & \mathrm{Ce}^{\cdot\cdot\cdot} & & & \end{array}$$

$$\begin{array}{llllll} & & & \mathrm{Ni}^{\cdot\cdot\cdot} & & \\ & & & \mathrm{Co}^{\cdot\cdot} & & \\ \mathrm{Cu}^{\cdot\cdot}, & \mathrm{Ag}^{\cdot}, & \mathrm{Zn}^{\cdot\cdot}, & \mathrm{Fe}^{\cdot\cdot}, & \mathrm{Tl}^{\cdot}, & \mathrm{Mn}^{\cdot\cdot\cdot} \\ & & & \mathrm{Cd}^{\cdot\cdot} & & \end{array}$$

The separation and identification of $\mathrm{Ti}^{\cdot\cdot\cdot\cdot}$, $\mathrm{U}^{\cdot\cdot\cdot\cdot}$, $\mathrm{Ce}^{\cdot\cdot\cdot}$, and $\mathrm{Tl}^{\cdot\cdot\cdot}$ was studied employing various developers. Titanium was distinguished from bismuth, silver, thallium, mercury, and copper by development with an alcoholic solution of benzoyl acetone slightly acidified with hydrochloric acid;

this gave a yellow-orange colour to the titanium band. To distinguish from iron, a neutral solution of the ketone was employed followed by diluted hydrochloric acid (1 in 20). The red-brown band at first produced descended the column leaving a yellow band in its place, the titanium complex being stable to acids while the iron reacts. The separation from chromium, thallium, and lead was imperfect.

For uranium, potassium ferricyanide was employed and gave a red-brown colour. Good separation was not obtained from arsenic or chromium. For thallium, the separation was generally imperfect. Ammoniacal potassium chromate gave a brown colour and dithizone in 4 per cent. sodium hydroxide solution, a cherry red.

With cerium, a solution of benzidine in acetic acid gave an intense blue colour.

A. H. Bennett

Physical Methods, Apparatus, etc.

Micro-effusiometry. L. K. Nash (*Anal. Chem.*, 1948, **20**, 258–262)—The apparatus described is for determining the molecular weight of 0·5 ml. of a permanent gas or 0·5 mg. of a volatile liquid.

Apparatus for gases—The manometer M (Fig. 1), containing distilled mercury, is 6 to 7 cm. high and is made from 24-mm. tubing. The bore of the tap S is at least 2 mm. in diameter. The right branch of the manometer is sealed to a length of capillary tubing. Sections B and D are 3-cm. lengths of 2-mm. tubing, sections C and E are 2-cm. lengths of 0·5-mm. capillary, and section F is a 6-cm. length of 2-mm. capillary. The bulb I contains the auxiliary manometer liquid, tetralin. The bulb O is prepared by blowing a thin-walled bulb, 0·01 to 0·02 mm. in thickness and 12 mm. in diameter, on the end of a length of 6-mm. tubing. A small orifice in the bulb is made by placing an earthed copper wire in the tube and a Tesla coil leak tester on the outside. The voltage of the discharge is increased until the discharge strikes through the bulb. The bulb is sealed to the apparatus at point X.

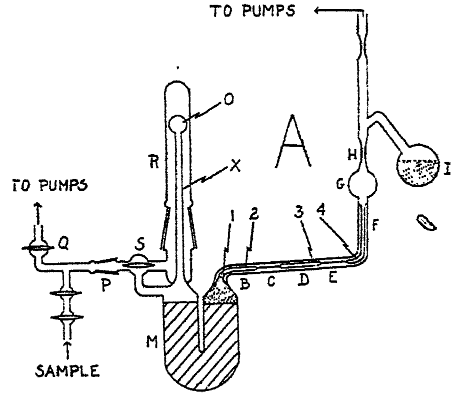


Fig. 1.

Standardisation—Evacuate both sides of the manometer. Distil tetralin from I into the right branch of the manometer until the tetralin level is close to point 1. Close the tap Q and turn S to connect P to M. Introduce about 0·5 ml. of dry nitrogen. The tetralin level should reach the region of point 4. Turn S to connect the outer space R to P and open Q. Measure the time taken for the tetralin meniscus to move from point 3 to point 2. Alter the amount of tetralin in the manometer until the time taken is 6 to 8 min. and then seal the right arm of the manometer at H. Repeat the introduction of nitrogen and measurement of the time for effusion until concordant results are obtained.

Procedure—Introduce about 0·5 ml. of the gas into the space over the left branch of the manometer, turn S to connect P to outer space R, open Q, and measure the time for effusion as above. Repeat several times. From the time of effusion for nitrogen, and using Graham's law, calculate the molecular weight of the gas.

The average error of a series of molecular weight determinations was 1 per cent.

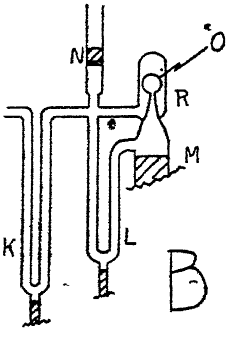


Fig. 2.

Apparatus for volatile organic liquids—This is illustrated in Fig. 2. The taps are replaced by mercury cut-offs and the joint in the outer jacket is eliminated. Introduce the liquid to the evacuated apparatus by touching the tip of a capillary pipette containing the sample against the surface of the fritted disc N, which is sealed against the atmosphere by a small quantity of mercury. Raise the mercury in the U-tube L, lower the mercury in K, and proceed as above. Benzene is a suitable liquid for use as a standard. The error of the estimation is usually not greater than 3 per cent.

B. Atkinson

Improved Horizontal Micro-burette. A. Lacourt (*Metallurgia*, 1948, **38**, 355–356)—To ensure a regular, slow, reproducible rate of flow with micro-burettes made from tubing up to 3·5-mm. bore, a very fine tip is necessary and this introduces difficulty in filling and cleaning and causes a variation in flow due to change in position.

Introduction of a 7- to 8-cm. vertical ascending capillary tube between the horizontal tube and the descending one gives a reproducible flow without the necessity for a very narrow tip, and the flow ceases when contact of the tip with the liquid or glass surface is broken. Mount the micro-burette on a board bearing a paper millimeter scale, and calibrate it by using either bi-iodate and thiosulphate or the solutions to be used subsequently. Clean the apparatus with water, ethanol, or dilute nitric acid, but not sulphuric acid or sulphuric-chromic acid, and allow it to dry before use; the apparatus must be cleaned and dried immediately after use.

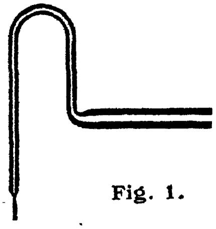


Fig. 1.

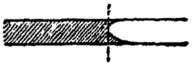


Fig. 2.

Fig. 1.—Construction of modified burette.
Fig. 2.—Meniscus in wide-bore tubing.

With instruments of wider bore, the meniscus takes a somewhat different form from that usually obtained, but good reproducibility is attained when the level is taken as shown in Fig. 2.

M. E. DALZIEL

Miscellaneous Microchemical Devices. XV. Device for Testing Gases. J. T. Stock and M. A. Fill (*Metallurgia*, 1948, **38**, 356)—The device is constructed from 2-mm. bore glass tubing, the guide-arm, A, being of glass rod and able to slide easily into the cap C. A strip of filter paper, D, impregnated with a suitable reagent is gripped between the gas-tube B and C, being held firmly in place by a rubber band, E. Gas penetrating the test paper escapes round A. Proper contact between the gripping tube-ends is ensured by rotating a small copper disc bearing carborundum on both sides between the ends, after they have been cut as cleanly and squarely as possible.

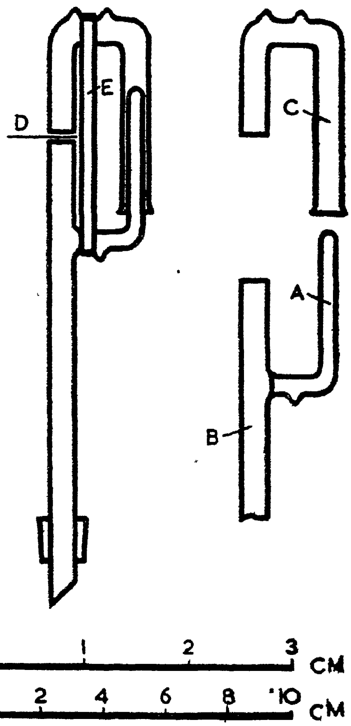


Fig. 1. Gas-testing apparatus (upper scale, micro version; lower scale, larger version)

M. E. DALZIEL

Reviews

PRACTICAL PUBLIC HEALTH PROBLEMS. By SIR WILLIAM SAVAGE, B.Sc., M.D. Second Edition. Pp. vii + 197. London: J. A. Churchill Ltd. 1949. Price 14s. net.

This well known book, the first edition of which was reviewed in *The Analyst* in 1940, deals with many of the problems of disease prevention which confront the Medical Officer of Health and the Sanitary Inspector. The first five chapters are devoted to water, sewage and effluents, including the disposal of effluents containing milk or whey, a special problem in Somerset. Then follow four valuable chapters on the bacteriological control of milk supplies and on the supervision and control of milk pasteurisation. The author's analysis of the available statistics on milk-borne tuberculosis amply justifies his description of the present position as "deplorable."

The chapter on the Food and Drugs Act, 1938, which appeared in the first edition, has been replaced by one on hygienic food control, which deals, *inter alia,* with slaughter houses, unsound food, ice-cream and communal eating places. The author lists the hygienic requirements that he regards as essential for restaurants, etc., and expresses the hope that the time will come when only those who have attended a suitable course will be eligible for employment in catering establishments.

A chapter on field investigation of food poisoning summarises the latest knowledge on this subject, and is full of valuable hints based on personal experience of the difficulties associated with these inquiries.

It is surprising that the inspection of canned foods, a subject to which the author has made such notable contributions in the past, is allotted only eight pages, but in his view the industry is now so scientifically organised that the amount of spoilage is small and the danger to health trifling.

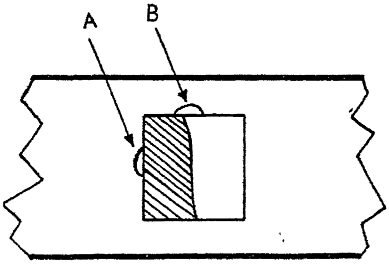
Other questions dealt with are the investigation of enteric and infectious disease outbreaks, housing inspection and disinfection. One activity that closely concerns the Medical Officer of Health—the control of household vermin—is not touched upon: some account of modern practice in this field would be welcome in future editions.

Sir William Savage expresses decided opinions on many matters, including such controversial points as the replacement of chemical by bacteriological analysis in water examination. His views may not always be accepted without question, but they represent the conclusions of long practical experience as County Medical Officer of Health. The book is of great value as a concise, interesting and informative survey by one of our foremost authorities on public health administration. G. W. MONIER-WILLIAMS

Mikro-Methoden zur Kennzeichnung Organische Stoffe und Stoffgemische. By **Ludwig Kofler** and **Adelheid Kofler**. Pp. viii + 337. Innsbruck: Universitätsverlag Wagner Ges.M.B.H. 1948. Price about 36s.

Instruction on how to identify organic "spots" formerly insisted that the student should first ascertain the characteristic elements present, then study the reactions of the substance to find groups of elements; if the substance was solid, the melting-point could then be determined and suitable derivatives prepared to clinch the identification. It must be admitted that many students were tempted and succumbed: they determined the melting-point FIRST and short-circuited the official procedure by reference to Clarke's *Handbook of Organic Analysis.* The reviewer has been told by some modern students that it is no longer thought necessary to defer the melting-point determination, but Drs. Kofler and Kofler have carried the tendency further than the most wishful student can have imagined. From a melting-point study, the identification of an unknown solid is almost completed.

The authors determine the melting-points on a microscope hot-stage, two types of which are described. In one the temperature is measured by means of a copper-constantin thermocouple, in the other a thermometer is used and is calibrated by observing compounds having known melting-points. The use of the melting-point as a test of purity is well known and the authors claim that, in general, their method will detect about 0·25 per cent. of impurity, whereas the capillary tube method will not show less than about 2 per cent. The purification of an impure substance, or the isolation of one component from a mixture, can be effected by heating until melting just begins and absorbing the eutectic melt (*e.g.*, on filter paper), repeating the procedure as many times as may be found to be necessary.



After the melting-point of an unknown substance has been determined a special "contact slide" is prepared, on which the unknown is in contact with a selected reference compound. To prepare this slide the higher melting of the two substances is fused and a drop placed at one edge *a* (see Fig.) of a square cover glass so that it runs below the latter. After this has solidified (as shown in Fig.) the melt of the lower melting substance is introduced at *b*. The junction of the two solids is observed through the microscope, using crossed Nicols, while the hot-stage is warmed gradually. If a eutectic is formed, a dark band of liquid is seen between the two slides and the melting-point of the eutectic may be determined. The reference compounds selected for use on the contact slide depend upon the melting-point of the unknown substance; two are used, separately, with each unknown. The complete list of reference compounds is as follows:—

M.p. of unknown	Reference compounds
20° to 100° C.	Azobenzene, benzil
100° to 120°	Benzil, acetanilide
120° to 140°	Acetanilide, phenacetin
140° to 170°	Phenacetin, benzanilide
170° to 190°	Benzanilide, salophene
190° to 340°	Salophene, dicyandiamide

It is obvious that all binary systems do not form simple eutectics, but this point is adequately covered by the authors, who illustrate and describe the usual phase-rule diagrams and reproduce photomicrographs showing the contact boundaries for a number of systems. The contact slide method is developed and it is shown how the micro-thermal analysis of a system is possible: three-component systems are also considered briefly.

Further information is obtainable by determining the molecular weight of the unknown substance, using as solvent bornyl chloride between slide and cover glass, or camphor in a capillary tube on a modified hot-stage. Greater use is made, however, of the estimation of the refractive index of the unknown substance in the liquid state. Glass powders of known refractive indices are immersed in the melt and the temperature is raised until examination under the microscope shows that the refractive indices of the glass and melt are equal. The refractive index range from 1·3400 to 1·6718 is covered by 23 glasses.

The authors have examined over a thousand organic compounds by these methods and have tabulated the results, the compounds being arranged in order of melting-point. The table includes the eutectic temperatures with the reference compounds, the refractive index of the melt and "special characteristics" (*viz.*, mode of crystallisation, occurrence of sublimates, polymorphs, hydrates, liquid crystals, etc.). It seems to the reviewer that the novel procedures described in this book could form a simple and very rapid means of identifying organic compounds: such a method would be welcome in industrial laboratories. However, it is realised that, in order for the application to be widespread, many more substances must be examined and the results tabulated for reference: L. and A. Kofler have done much work in compiling their table, but this must be extended.

Readers in this country may be disappointed to find that a book of this price should be bound in paper covered boards, only the spine being cloth, but the binding appears to be strong. The text and illustrations are clearly printed on good quality paper. The book is adequately indexed and a separate index is provided for the substances in the table. There are 204 references to the literature.

G. H. Wyatt

BIOLOGICAL METHODS GROUP

A Joint Meeting of the Group with the Society for Endocrinology will be held on Thursday, October 20th, 1949, at the Medical Society of London, Chandos Street, Cavendish Square, W.1, from 2.15 p.m. until 7 p.m. The meeting will take the form of a symposium on Hormone Assay, and the Chair will be taken by Professor J. H. Gaddum, F.R.S.

The following aspects of the subject will be presented and discussed:—

(1)	General Approach to Biological Assay	Dr. H. O. Schild.
(2)	Assay of Thyroxine and Thyroidal Activity	Dr. G. F. Somers.
(3)	Assay of Insulin	Mr. K. L. Smith.
(4)	Assay of Gonadotropins	Dr. J. A. Loraine.
(5)	Assay of Posterior Pituitary Extracts	Mr. G. A. Stewart.
(6)	Clinical Methods of Assaying Oestrogens	Dr. P. M. F. Bishop.

Light refreshments will be available at 4 p.m.
Visitors will be welcome.

METHODS OF WATER ANALYSIS

In 1947 a Joint Committee representing the Institution of Water Engineers, the Royal Institute of Chemistry and the Society of Public Analysts and Other Analytical Chemists was set up by the Institution of Water Engineers for the purpose of standardising methods of water analysis.

The Report of this Committee laying down standard methods for the physical and chemical examination of potable waters is shortly to be published in the Journal of the Institution of Water Engineers. Reprints of this Report will be available to members of the Society, price 3s. 6d., post free. Application should be made, with remittance, and not through Trade Agents, to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

SEPTEMBER, 1949 Vol. 74, No. 882

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

The Chemical Determination of Nicotinic Acid in Food Products

By P. O. DENNIS and H. G. REES

(*Read at the meeting of the Society on Wednesday, May 4th,* 1949)

CHEMICAL methods for the determination of nicotinic acid in the quantities normally found in foodstuffs are based on the reactivity of the pyridine ring, rupture of which is relatively easy. The most satisfactory reagent is cyanogen bromide and the observation of König[1] that a yellow colour is developed when pyridine is treated with cyanogen bromide in presence of an aromatic amine has been adapted by many workers[2,3,4,5] for the colorimetric estimation of nicotinic acid. The choice of amine is important for the development of the colour, particularly with regard to time for maximum intensity and stability.[6,7,8] Another step in the procedure that has received much attention is the preparation of the solution to be analysed; interfering substances must be removed and the solution rendered colourless or nearly so. Procedures proposed include acetone precipitation,[9] permanganate oxidation[10,11] and adsorption techniques.[7] Permanganate oxidation has proved suitable for urine analysis, but we find it to be unsatisfactory with meat products and James *et al.*[7] report indifferent results with cereals. For the past five years we have used a modification of Bandier's acetone method[9] on all materials when a purification is necessary. This is rapid and sufficiently accurate for all routine analysis, and it appears appropriate to publish the following results for workers in this field.

REAGENTS—

Nicotinic acid primary standard—Dissolve 50 mg. of nicotinic acid in 50 per cent. alcohol and make up to 200 ml. with the same solvent. This solution is stable for many months if stored in tightly-stoppered bottles in the refrigerator.

Nicotinic acid secondary standard—Prepare daily by diluting 10 ml. of primary standard to 250 ml. with water. It contains 10 μg. per ml.

Phosphate buffer solution—A 2 per cent. solution of AnalaR potassium dihydrogen phosphate in distilled water. The pH is 4·6 and the solution is stable for long periods in a refrigerator.

Cyanogen bromide reagent—Make an approximately 4 per cent. aqueous solution of cyanogen bromide by just decolorising ice-cold saturated bromine water with chilled 10 per cent. sodium cyanide solution. The bromine water and sodium cyanide solution should be made up every few days and stored in a refrigerator and the cyanogen bromide prepared just before use. Care must be taken to avoid an excess of sodium cyanide in the final solution.

Procaine hydrochloride solution—This substance is obtainable in a highly purified state for medicinal use and such material is eminently suitable as a reagent. Dissolve 3·5 g. in

80 ml. of water, add 1·5 ml. of AnalaR concentrated hydrochloric acid and make up with water to 100 ml. The solution is stable in light and air, but is best made up daily.

Acetone—Redistilled and dried over calcium oxide.

Kieselguhr—Commercial Supercel is washed with water and dried.

COLORIMETER—

A sensitive photo-electric colorimeter is recommended such as the Gallenkamp direct-reading instrument with a galvanometer calibrated logarithmically to give readings of the extinction coefficient $E_o \times 100$. A suitable filter for the colour measurement is Ilford Micro 1 Blue.

PROCEDURE—

We have found that certain materials such as meat extracts and pharmaceutical products not containing cereals and containing more than 500 µg. of nicotinic acid per gram yield light-coloured solutions after hydrolysis and neutralisation, thus enabling a simplified technique involving no solvent extraction to be used. Weigh out a quantity of the material containing between 1500 and 2500 µg. of nicotinic acid and digest it with 25 ml. of 4 *N* caustic soda on the steam-bath for 30 to 40 minutes. Cool and adjust to pH 4·0 with concentrated hydrochloric acid, using bromophenol blue as an external indicator; cool, make up with water to 100 ml. and filter through sintered glass to obtain a bright solution. The filtrate contains between 15 and 25 µg. of vitamin per ml. and, although coloured, is suitable for analysis without further treatment. One-ml. aliquots of this test solution are taken for analysis. The following mixtures are set up, preferably in 25-ml. volumetric flasks:

	T_1 ml.	T_2 ml.	S_1 ml.	S_2 ml.	S_3 ml.
Test solution	1·0	1·0	—	—	—
Nicotinic acid solution, 10 µg./ml. ..	—	—	1·5	2·0	2·5
Water	1·5	1·5	1·0	0·5	—
Phosphate buffer solution, pH 4·6 ..	5·0	5·0	5·0	5·0	5·0

Heat the flasks in a water-bath to 60° C. and add to each 2 ml. of cyanogen bromide reagent, continue heating for 15 minutes, remove, and cool to room temperature. Add 10 ml. of procaine reagent to T_1 and T_2, make up to 25 ml. with water and measure the colour in a photo-electric colorimeter within 2 to 3 minutes, for under these conditions colour development is almost instantaneous. To flasks S_1, S_2 and S_3 add 10 ml. of procaine reagent followed by 1 ml. of the test extract, make up to 25 ml. with water and measure the colour intensity.

Plot a graph of the galvanometer readings (*i.e.*, $E_0 \times 100$) of S_1, S_2 and S_3 against the nicotinic acid contents of the tubes, *viz.*, 15, 20 and 25 µg. This should give a straight or very nearly straight line. From this the nicotinic acid contents of T_1 and T_2 may be read directly. By using this method of computation the necessity of preparing solution and reagent blanks, such as are required by the usual increment methods as employed by Wokes, is avoided, because corrections for the inherent colour of the test solution and for colour development between the extract and amine have been made by adding the equivalent amount of the extract to S_1, S_2 and S_3 after colour development and just before making up to 25 ml. Our experience has shown that no appreciable colour develops between the extract and the amine or by heating the extract with cyanogen bromide. Further, the accuracy of the increment method depends upon a strict adherence to Beer's law, a deviation from which is observed in the higher ranges of concentration of nicotinic acid.

For materials that have a low nicotinic acid content and yield highly coloured solutions after hydrolysis and neutralisation the following procedure is adopted. Weigh out sufficient material to contain between 250 and 500 µg. of the vitamin and digest with 25 ml. of 4 *N* caustic soda or 15 ml. of concentrated hydrochloric acid. Meat and yeast products are best hydrolysed with alkali, but acid is preferable for cereal products. These conditions yield lighter coloured solutions for analysis and simplify the technique of hydrolysis. In certain cases, *e.g.*, with corned beef, we have employed mild hydrolysis under pressure. After hydrolysis, cool, neutralise to pH 4·0 and make up to 50 ml. Take 5-ml. aliquots in each of two 50-ml. centrifuge tubes and treat both in the following manner to yield duplicate test solutions. Add 0·8 to 1·0 g. of kieselguhr and mix thoroughly, run in 9 to 10 volumes of dry acetone from a burette with constant stirring, close with a rubber bung and mix by vigorous shaking. The precipitate should be of a light fluffy nature; if it is not, insufficient kieselguhr has been used. Centrifuge and decant the supernatant liquid from each tube into an 150-ml. volumetric flask, add two or three pieces of "bumper" and evaporate off the acetone under vacuum to a volume of about 5 ml., warming occasionally by immersion in warm water. To the residue in the centrifuge tube add a further 9 to 10 volumes of acetone, re-extract, centrifuge, add the supernatant liquid to the corresponding volumetric flask, and re-evaporate until there is no obvious odour of acetone in the flask, that is, to about 2 or 3 ml. Transfer the contents quantitatively to a 20 or 25-ml. flask with successive small portions of phosphate buffer solution, make up to volume and filter through a sintered glass funnel to obtain a bright test solution containing between 1 and 2 μg. of vitamin per ml. Set up the following mixtures in 25-ml. volumetric flasks.

	T_1 ml.	T_2 ml.	S_1 ml.	S_2 ml.	S_3 ml.	S_4 ml.
Test solution	5·0	5·0	—	—	—	—
Nicotinic acid solution, 10 μg./ml. ..	—	—	0·5	0·5	1·0	1·0
Water	1·0	1·0	0·5	0·5	—	—
Phosphate buffer solution, pH 4·5 ..	—	—	5·0	5·0	5·0	5·0

Heat these mixtures to 60° C., add the cyanogen bromide and develop the colour exactly as before. From the average readings of S_1 and S_2 and of S_3 and S_4 a graph is plotted, and from it the nicotinic acid contents of T_1 and T_2 are read directly. We have usually found that the acetone precipitation yields a nearly colourless test extract and that on diluting 5 ml. to 25 ml. a negligible blank is produced; we also find it unnecessary to prepare a reagent blank if acidified procaine and phosphate buffer are used. If the test extract should be coloured, then a correction may be applied by adding 5 ml. of test solution to tubes S_1, S_2, S_3 and S_4 after the addition of procaine and just before making up to volume and reading the colour.

CALIBRATION CURVES—

From the many calibration curves obtained when studying the conditions necessary for optimum colour development, the following table gives an indication of the type of curve obtained with pure nicotinic acid. The conditions for colour development were essentially those recommended in the methods described above.

μg. Nicotinic acid	$E_0 \times 100$
5	9·0
10	17·3
15	25·8
20	34·0
25	42·0
30	49·3
35	54·5
40	60·0

RECOVERY EXPERIMENTS—

Before the method was applied to food products, numerous recovery experiments were carried out with known quantities of nicotinic acid added to protein hydrolysates and composite meat and yeast products. Without quoting these results in detail we may state that satisfactory recoveries of the order of 98 to 101 per cent. were always obtained.

NICOTINIC ACID CONTENT OF VARIOUS FOOD PRODUCTS—

As an indication of the validity of the method we quote the following results obtained on duplicate samples of the products analysed by Wokes.[7] The results given in column 1 were obtained by our technique and those in columns 2 and 3 are taken from Wokes's paper.

	Nicotinic acid in μg./g.		
	Chemical assay		Microbiological assay
	1	2	3
Meat extract	1050	1027	1082
Yeast extract	560	478	537
Dried yeast	300	332	331
Malted barley	105	126	127
Wheat germ	135	134	129
Malt extract	96	102	95

Finally we append a series of results obtained on a variety of meat products over an extended period.

	Total creatinine, %	Nicotinic acid, µg./g.
Meat extract:		
(*a*)	7·80	1151
(*b*)	7·80	1124
(*c*)	7·52	900
(*d*)	7·45	870
(*e*)	7·40	890
(*f*)	7·00	810
Desiccated beef powder:		
(*a*)	1·22	126
(*b*)	0·90	102
(*c*)	0·90	117
(*d*)	0·88	135
Meat peptones:		
(*a*)	—	160
(*b*)	—	148
(*c*)	—	190
Meat juice:	Water	
(*a*)	58·0	362

	Water, %	Fat, %	Nicotinic acid in µg./g.	Nicotinic acid in µg./g. (dry wt. and fat-free)
Corned beef:				
Dry cure				
(*a*)	56·4	14·0	49·5	161·0
(*b*)	55·3	18·3	39·7	150·5
(*c*)	55·0	16·0	37·9	130·6
(*d*)	57·4	13·7	29·6	100·5
Wet cure				
(*a*)	64·9	2·9	40·7	126·5
(*b*)	58·4	10·4	35·7	114·5
(*c*)	53·8	14·7	29·5	93·0
(*d*)	55·8	6·8	29·5	78·7

SUMMARY

A colorimetric method involving reaction with cyanogen bromide and coupling with procaine for the determination of nicotinic acid in food products has been presented. Rigorous purification of the extracts and compensation for reagent and extract blanks have been reduced to a minimum and are unnecessary for materials rich in nicotinic acid. To minimise the colour of the test extract, acid hydrolysis is recommended for cereals and alkaline hydrolysis for meat and yeast products.

The authors wish to thank the Directors of Oxo Limited for permission to publish these results, and to Dr. E. C. Wood for criticism of the manuscript.

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Discussion

Dr. W. F. Elvidge asked if it had been found necessary to adjust the pH of the cyanogen bromide reagent. He agreed with the authors in preferring procaine to *p*-acetophenone. He agreed also that it was necessary to make calibration curves day by day at the same time as the assays. Sometimes better results were obtained with the Beckmann instrument than with the absorptiometer plus filter. Difficulty had sometimes been experienced in obtaining a clear filtrate after addition of cyanogen bromide.

Miss C. Klatzkin stated that experience gained during thousands of assays of nicotinic acid in cereal products confirmed the authors' findings as to the advantage of acid extraction. However, even when an adsorption technique was subsequently applied the interfering substance might not be completely removed, and a blank was found helpful. The adsorption technique did, however, remove a good deal of interfering substance, as shown by the colourless solutions obtained, and in her experience did not take any longer to carry out than procedures such as that described by the authors. One of the causes of the high amine blanks she had encountered was found to be the presence of furfural. Did the authors' method obviate this difficulty without recourse to blanks? In regard to the stability of the colour given with procaine, an earlier study on cereal products by James, Norris and Wokes (*Analyst*, 1947, **72**, 327) had shown that the maximum intensity was reached in about 10 minutes after mixing, and by 15 minutes fading had set in. Timing was perhaps not so critical as with *p*-aminoacetophenone, but should not be ignored.

Mr. J. King, in answer to a question on the use of cation exchange zeolites such as Decalso for separating nicotinic acid from interfering substances, particularly amino acids and their addition products, suggested that theoretically it would be sounder to try the use of anion exchange resins of the zeo-carb 215 or 216 types containing .COOH or .SO_3H groups, as used by Consden, Gordon and Martin for the separation of acidic amino acids and by Bendall, Partridge and Westall for the separation of bases and amino acids (*Nature*, 1947, **160**, 374).

Dr. D. W. Kent-Jones said that his own earlier experiences with nicotinic acid assays using chemical methods and that of the Society's Sub-Committee working on the subject had been disappointing, and he was pleased to hear of a method for which a claim was made that it worked. Nevertheless, in view of previous disappointments, he would not be convinced of its usefulness until he had had experience of it himself.

He mentioned that arising from the work of the Sub-Committee, a note had been published in *The Analyst* (1947, **72**, 501) pointing out that some specimens of nicotinic acid contained *iso*-nicotinic acid and this complicated the problem as *iso*-nicotinic acid was not biologically active and should not be included in the assay.

Mr. W. B. Emery asked if the authors had any experience of the use of anion exchange resins in the purification of extracts for the assay. He would not expect very close agreement between the results of chemical and microbiological assays, since naturally-occuring compounds existed that gave the reaction, but had no biological activity.

Mr. J. Haslam asked if any degree of control of "room temperature" was advisable in the development of colour.

Mr. Dennis, in answer to the questions, agreed that the Beckmann spectrophotometer could be used with advantage. With regard to *iso*-nicotinic acid no great differences were observed in curves based on highly purified nicotinic acid and pharmaceutical specimens. It was also found that in presence of phosphate buffer, and under the conditions stated, no colour was produced with *iso*-nicotinic acid. The results obtained did agree with microbiological assays. When using *p*-aminoacetophenone, the very careful timing mentioned in Wokes's[7] paper was found necessary. Cyanogen bromide did not affect the pH in presence of the phosphate buffer. The authors had not been primarily concerned with cereals and had encountered no interference due to furfural. Exchange resins had not been tried as a means of purification. Provided that both test and standard tubes were cooled to "room temperature" at the same time, small differences in the final temperature were not important; however, the greater the degree of control over all the reaction conditions, the more closely did readings of successive standard curves agree.

The Use of Ether Extraction in the Determination of Uranium

By T. R. SCOTT

Although ether extraction of uranyl nitrate is now extensively used in the determination of uranium, such details of the technique as have appeared in print are scarcely adequate as a guide to the analyst. In particular, it is desirable to know to what extent and in what ways the extraction conditions may be varied without diminishing the usefulness of the method. It is the purpose of this paper to supply these details and, in addition, to describe an extraction procedure suitable for general application.

EXPERIMENTAL

Experiments have been conducted by shaking aqueous solutions of known composition with ether of "anaesthesia grade," measuring the volumes of the two layers after shaking has been completed and determining the composition of the ether layer by the hydrogen peroxide method (q.v.). Since the work was designed specifically to provide information for control of analytical procedures, the time of shaking was confined in all instances to 1 minute and no claim is made that results represent accurately controlled equilibrium conditions. It is nevertheless known that equilibrium is attained very rapidly in experiments of this type and the fact that repeated determinations of "percentage uranium extracted" generally agree within ± 1 per cent. is sufficient indication that only small errors are introduced by restricting the time of shaking.

The extent to which extraction of uranyl nitrate has occurred is normally expressed by the partition coefficient, *i.e.*, the ratio of the equilibrium concentration in the ether layer to that in the water layer. With high partition coefficients, however, small experimental errors are unduly magnified and results in the present work are preferably expressed as the percentage of uranium extracted from the aqueous layer, when equal volumes of both layers are present after shaking. Then,

$$\text{Percentage Extracted} = \left(\frac{\text{Partition coefficient}}{1 + \text{Partition coefficient}}\right) \times 100$$

Variables that have been considered include temperature, nature and concentration of salts, concentration of nitric acid and uranyl nitrate, nature of interfering radicals and extraction of nitric acid. These will be discussed in turn.

TEMPERATURE—

Effects of temperature, over the usual range of room temperatures, are inconsiderable and could not be detected within the limits of error of the experimental method.

NATURE OF INORGANIC NITRATES PRESENT—

Extraction of uranyl nitrate by ether is negligibly small unless the water layer contains quantities of inorganic nitrates or nitric acid or both. A comparison of the effects of various salts (at the same nitrate ion concentration) is made in Table I. Ferric nitrate, being most efficient in salting-out uranyl nitrate, has been used in the present work and has the additional advantage of reducing interference due to phosphate ion.

TABLE I

EFFECT OF INORGANIC NITRATES USED IN ETHER EXTRACTION

Salt	Concentration of the salt	Acid concentration	Percentage of uranium extracted
NH_4NO_3	3·0 *M*	1·5 *N*	30
$NaNO_3$	3·0 *M*	1·5 *N*	38
$Ca(NO_3)_2$	1·5 *M*	1·5 *N*	54
$Mg(NO_3)_2$	1·5 *M*	1·5 *N*	62
$Ce(NO_3)_3$	1·0 *M*	1·5 *N*	57
$Fe(NO_3)_3$	1·0 *M*	1·5 *N*	69

CONCENTRATION OF FERRIC NITRATE AND NITRIC ACID—

For analytical purposes it is essential to know the number of extractions required to recover (say) 99 per cent. of the uranium from a given solution (the actual percentage to be removed varying with the degree of accuracy of the work). Knowledge of the effects of salt and acid concentrations on the partition coefficients is thus of great importance, especially as the range of variation is considerable. These two variables have consequently been examined in detail and the results are shown in Fig. 1.

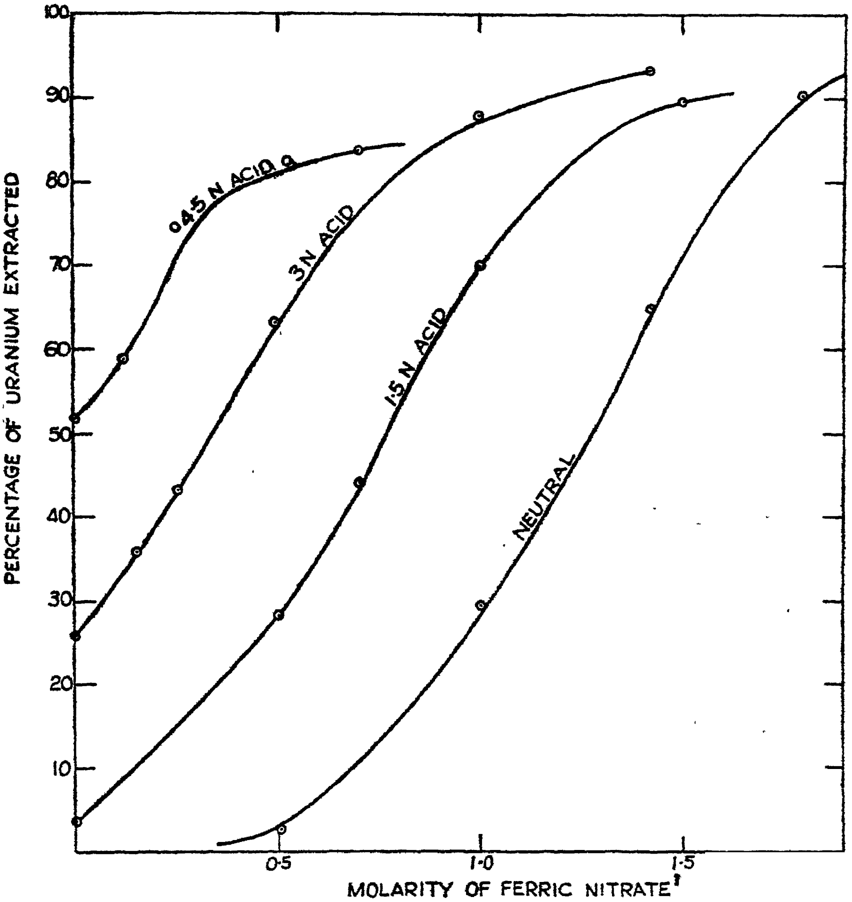

Fig. 1. Variation in percentage of uranium extracted with change in nitric acid and ferric nitrate concentrations

With 4·5 N acid erratic values were obtained for the percentage of uranium extracted, particularly at the higher concentrations of ferric nitrate, and it is therefore recommended

TABLE II

VARIATION IN CONCENTRATION OF URANYL NITRATE

Nitric acid concentration	Ferric nitrate concentration	U_3O_8 concentration %	Percentage extracted
3·0 N	1·0 M	1·1	82·5
3·0 N	1·0 M	0·7	83·8
3·0 N	1·0 M	0·2	87·6
3·0 N	1·0 M	0·05	85·7
3·0 N	1·0 M	0·01	90·0
3·0 N	1·0 M	0·003	88·8

that acid concentrations should not exceed 3 *N* in the aqueous layer prior to extraction. With stronger acid, noticeable reaction with the ether also occurs.

CONCENTRATION OF URANYL NITRATE—

Table II shows the variation in percentage extracted over a range of concentrations. The variations are not regarded as being of importance in analytical operations.

INTERFERING RADICALS—

Anions—Although it is customary to extract uranyl nitrate from solutions containing nitrate as the only anion, large concentrations of other anions can be tolerated without modification of the routine procedure described below.

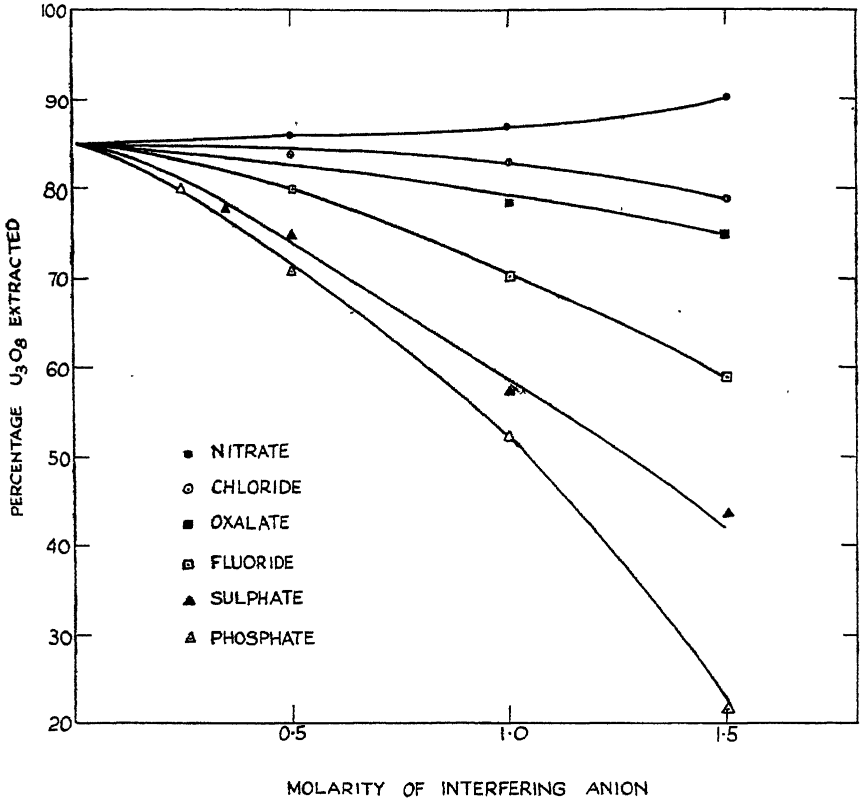

Fig. 2. Effect of various anions on percentage of uranium extracted

Solutions containing varying quantities of the common anions, introduced as the sodium salts, were made 3 *N* with nitric acid and 1 *M* with respect to ferric nitrate, and the percentage of uranium extracted was determined in the usual way. Results are shown in Fig. 2, from which it is evident that only fluoride, sulphate and phosphate can be regarded as seriously interfering radicals. The effect of acetate (not shown in the figure) is much the same as that of chloride. It should also be noted that, because of the solubility of ferric chloride in ether, significant amounts of this salt pass to the ether layer in presence of chloride ion.

Provided that the presence of foreign anions does not reduce the percentage of uranium extracted below 78·5 per cent., no modification of the routine procedure (q.v.) is required to achieve 99 per cent. recovery of uranium. The same recovery can be obtained by increasing the number of extractions to four if the percentage extracted lies between 78·5 and 68·4 per cent. With greater interference, it is generally preferable to reduce the concentration of the offending anion by precipitation with CO_2-free ammonia and solution of the precipitate in nitric acid before proceeding with ether extraction.

Cations—Thorium, zirconium, cerium (quadrivalent) and vanadium are extracted to a certain extent by ether, but the interference can be dealt with by subsequent modifications of the colorimetric method for uranium (q.v.). Vanadium, zirconium and titanium can also be completely separated from sexavalent uranium by precipitation with cupferron in acid solution, the filtrate containing only the uranium. The method is less satisfactory for thorium and cerium, precipitation being incomplete except in very dilute acid. None of the other cations generally encountered in uranium analyses cause interference in the method under discussion.

EXTRACTION OF NITRIC ACID—

Most of the variables considered above also influence the degree to which nitric acid is extracted from the aqueous layer. It is thus necessary to determine partition coefficients for nitric acid, since, in repeated extractions of the same aqueous solution, the nitric acid concentration and therefore the partition coefficient for uranyl nitrate both decrease. In practice, this effect is best controlled by adding to the aqueous layer, after each extraction, an amount of concentrated nitric acid equal to that extracted. A sufficient idea of the quantities of acid to be added can be obtained from Fig. 3 which gives the same information for nitric acid as Fig. 1 does for uranyl nitrate.

ANALYTICAL PROCEDURE

Details of a satisfactory routine procedure are given below, the italic letters in parentheses referring to the discussion which follows this section.

(1) Add a suitable aliquot (*a*) of uranium solution to a separating funnel, after ensuring that the solution is 3 *N* with respect to nitric acid and 1 *M* with respect to ferric nitrate (*b*).

(2) Add an equal or slightly greater volume of ether (*c*) to the funnel and shake, with periodic releases of ether vapour, for 1 minute. Allow 1 minute for settling and drain the aqueous layer into a small beaker.

(3) Swirl the ether layer around the inside of the funnel, allow to stand for 1 minute, and drain off the small amount of aqueous layer that has collected. Transfer the ether layer to a beaker containing 30 to 50 ml. of water (*d*).

(4) Return the aqueous layer to the funnel, rinse out the beaker with the calculated (*e*) quantity of nitric acid and add this to the funnel also. Proceed with a second and third extraction (*b*) following steps (2) to (4) in each instance.

(5) Stand the beaker, containing accumulated ether layers and water, in the draught of a fume-cupboard until all the ether is evaporated (*f*).

(6) Neutralise the residual aqueous solution with sodium hydroxide, using a small piece of litmus paper as indicator, and add sufficient nitric acid to make the solution just acid (*d*). Add sodium carbonate to give a final solution containing between 1 and 5 per cent. of Na_2CO_3.

(7) To the alkaline solution add a few pieces of porous pot, and heat on the hot plate to remove dissolved ether. Finally, boil for 15 minutes (*g*) and allow to cool to room temperature.

(8) Make the cold solution up to the required volume (*a*) and filter through a Whatman 542 paper. Using a portion of the filtrate as a blank, add to another portion 0·1 ml. of 30 per cent. hydrogen peroxide per 10 ml. of solution, and measure the uranium colour photometrically (at 360 mμ. with the Coleman spectrophotometer or at 434 mμ. with the Pulfrich photometer). In the presence of Zr, Ce^{IV} and Th, the procedure requires modification (*h*).

DISCUSSION

(*a*) It is convenient for solutions to contain 1 to 20 mg. of U_3O_8 when uranium is to be estimated finally by the peroxide colorimetric method. The final volume of solution (step 7) is then brought to 50 ml. for the lower, and 200 to 250 ml. for the higher, concentrations. In this way the colours developed fall within the range 2 to 10 mg. of U_3O_8, which is most suitable for measurement with the photometer. By using a greater optical depth of solution in the Coleman spectrophotometer (with a different type of cuvette), and employing a final volume of solution as small as 20 ml., it is possible to detect 0·02 mg. of U_3O_8, though such an amount cannot be measured with accuracy. With the Pulfrich photometer, 0·1 mg. can be detected.

(*b*) The recommended concentrations of ferric nitrate and nitric acid need not be rigidly adhered to, but for other concentrations it is necessary to work out from Figs. 1 and 2 the number of ether extractions and the amount of acid required in the course of the analysis. With the recommended concentrations, however, it is established that three extractions give 99 per cent. recovery for certain. No account is taken of other nitrates present in solution, but they help to increase the percentage of uranium extracted and ensure that the routine procedure gives even better than 99 per cent. recovery.

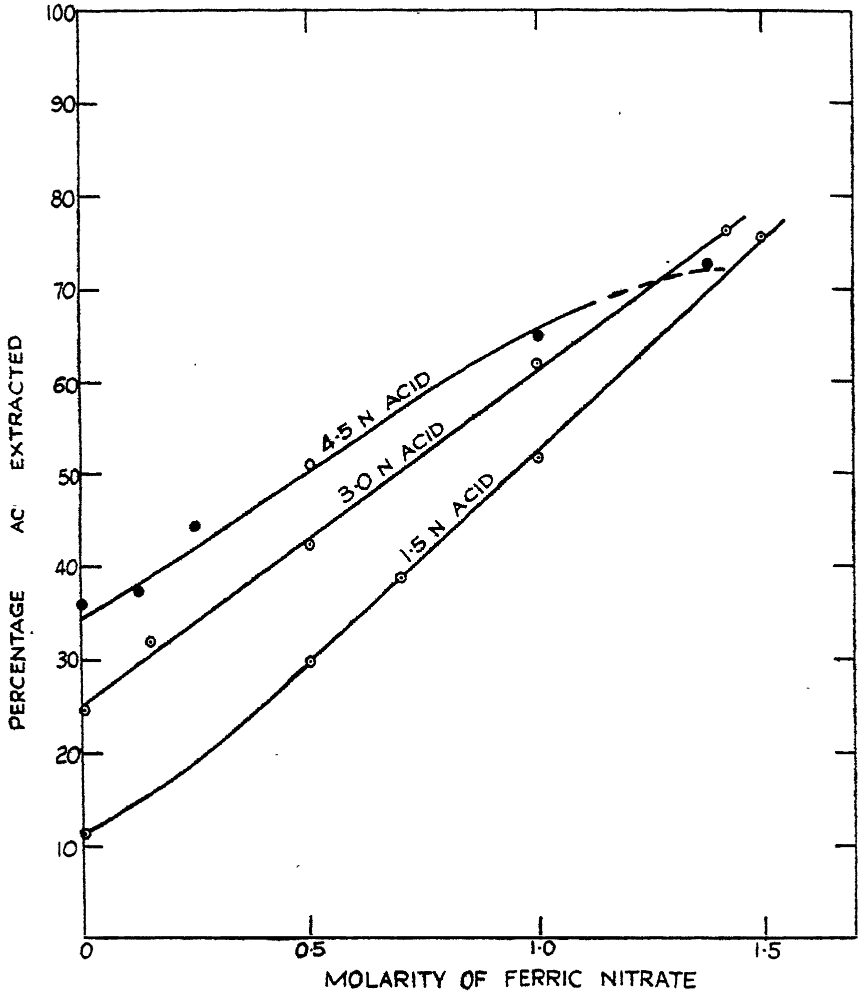

Fig. 3. Variation in percentage of nitric acid extracted

(*c*) Before the second and third extractions the beaker which has contained aqueous extract (step 4) is rinsed with the ether to be used for the next extraction. It is advisable to use a volume of ether slightly greater than that of the initial aqueous layer, so that, after shaking, the volume of the ethereal layer will not be less than the volume of the aqueous layer, despite losses due to evaporation and solution of ether in water. This ensures that efficiency of extraction is not less than that predicted by use of Figs. 1 and 3.

(*d*) If the acid ether extract is evaporated over sodium carbonate solution instead of water, bicarbonate may form and lead to large errors in the colorimetric estimation. Moreover, once sodium bicarbonate is formed under these conditions, it cannot be completely decomposed even with prolonged boiling. The technique of step (6) ensures that this source of error is eliminated. An alternative procedure is to measure the intensity of the yellow colour in the range 440 to 450 mμ., where the adsorption is not affected by the presence of bicarbonate; the sensitivity, however, is much reduced at this wavelength.

(*e*) Using the recommended concentrations of salt and acid, 1·2 ml. of nitric acid (sp.gr. 1·41) for every 10 ml. of uranium solution should be added before the second and subsequent extractions, to restore the normality of the aqueous layer to 3 *N*. For other concentrations the quantity of acid can be estimated from Fig. 1.

(*f*) This method is rapid and convenient and eliminates the need for heating the ether layer. It is advantageous if the draught can be augumented by lowering the sash of the fume-cupboard.

(*g*) Boiling has the effect of increasing the rate of coagulation of small quantities of ferric hydroxide, etc., which may be present. These impurities are not generally extracted by the ether, but are introduced by admixture of small amounts of the aqueous layer, an effect very difficult to avoid. The quantities of precipitate are so small, however, that solutions can be made up to the desired volume before filtering, without causing significant errors. It is often useful to add 1 ml. of 30 per cent. hydrogen peroxide before boiling to ensure the precipitation of impurities such as manganese, but boiling must then be continued for 45 minutes to decompose completely the peruranate formed.

(*h*) Thorium and cerium may be separated from uranium by adding hydrogen peroxide (in step 6) before making the solution alkaline with sodium carbonate. Under these circumstances only uranium remains in solution and may be determined as described in steps (6) and (7) of the procedure. If peroxide be added to the alkaline solution complete precipitation of thorium, etc. either does not occur or occurs very slowly.

Vanadium is also extracted by ether, despite the large excess of iron salts in solution, and develops a yellow colour with alkaline peroxide. Under the conditions of the recommended procedure, however, the partition coefficient for vanadium is approximately 50 times less favourable than that for uranium—consequently the error introduced when equal quantities of both are present is only slightly greater than 1 per cent., and there are likely to be many instances where the error is no greater than the error inherent in the colorimetric method.

SUMMARY

The variables affecting the ether extraction of uranyl nitrate from aqueous solutions have been examined and an analytical procedure capable of wide application has been described. Extractions are carried out in solutions containing 3 *N* nitric acid and 1 *M* ferric nitrate, the latter being a more efficient salting-out agent than the majority of inorganic nitrates. Under these conditions 99 per cent. recovery of uranium can be achieved by three extractions using equal volumes of ether and aqueous solution, and considerable amounts of other anions can be tolerated in solution without substantial modification of the procedure.

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
DIVISION OF INDUSTRIAL CHEMISTRY
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Use of the Van Slyke - Neil Manometric Apparatus for the Determination of Organic and Inorganic Carbon in Soil and of Organic Carbon in Soil Extracts

By J. M. BREMNER

INTRODUCTION

IN the course of a study of the chemistry of soil organic matter it became necessary to carry out numerous determinations of organic and inorganic carbon in soil and of organic carbon in soil extracts. This paper arose from a consideration of the methods available for these purposes.

Various wet and dry combustion methods have been used for the determination of organic carbon in soil, but the report of the Organic Carbon Committee of the International Society of Soil Science,[1] published in 1935, made it clear that dry combustion methods were the more reliable. The Committee found that the values obtained by a variety of dry combustion methods on nine different kinds of soil were so concordant that any choice between these methods could be made solely on grounds of laboratory convenience. The constant stream of papers on alternative methods of determining organic carbon in soil shows that although dry combustion in oxygen or air must be regarded as the standard procedure many workers on soil problems are dissatisfied with the method. The objections generally raised are that the apparatus required is expensive and occupies too much laboratory space, and that the method is too slow to be employed when a large number of samples are to be analysed. Rickson[2] recently described a modification of the Ter Meulen method for the determination of organic carbon in soil which is not so open to criticism on these points, since it can deal accurately with quantities on the semi-micro scale and is faster than the macro-methods, and the apparatus required is much more compact than those commonly used.

Various rapid titration methods[3,4,5,6] of determining organic carbon in soil have been developed which yield useful results when corrected by an appropriate factor. Unlike dry combustion procedures these methods need no modification for soils containing carbonates and can discriminate to some extent between relatively inert carbon in materials such as coal, charcoal or graphite and carbon in soil organic matter. Their use is limited, however, to comparison of related soils; they lack the precision necessary for investigations on the nature of soil organic matter.

Wet combustion methods, such as digestion of the soil with chromic and sulphuric acids, have proved unsatisfactory in the past owing to the low recoveries obtained. In only one of the wet methods tested by the Organic Carbon Committee of the International Society of Soil Science was the treatment drastic enough to achieve nearly complete oxidation of soil carbon to carbon dioxide. Some time after the Organic Carbon Committee's report was published, Van Slyke and Folch[7] studied the effect of adding various catalytic and oxidising agents to chromic acid mixtures and found that theoretical yields of carbon dioxide were obtained from all types of organic substances tested when a mixture of chromic, iodic, sulphuric and phosphoric acids was used for combustion. They employed the Van Slyke - Neil manometric apparatus[8] to collect and measure the carbon dioxide liberated on combustion and found that no modification of the method was required by the presence of any of the substances that interfere with, or require modification of, the usual dry combustion methods (*e.g.*, nitrogen, sulphur, halogens).

Since the Van Slyke and Folch wet combustion method was successful even with such difficultly oxidisable substances as cholesterol and palmitic acid, it seemed reasonable to expect that it would be applicable to the determination of organic carbon in soil. As shown below, this expectation was fulfilled. The method also proved to be suitable for the determination of carbon in soil extracts, for which no satisfactory method has previously been available. Moreover, it was found that the Van Slyke - Neil apparatus could be employed for the rapid and accurate determination of inorganic carbon in soil.

EXPERIMENTAL

Samples of the nine soils used in the investigation conducted by the Organic Carbon Committee of the International Society of Soil Science, and by Rickson to test his modified

Ter Meulen dry combustion method, were fortunately available in this laboratory. These samples were employed in the work described below as they represent a wide variety of soils and include some known to provide special analytical difficulties. All samples were finely ground and passed a 100 B.S. mesh. The moisture contents of the soils were determined by drying samples in an oven at 105° C. for 18 to 20 hours. The reactions involved in the determinations were all carried out in combustion tubes attached to the chamber of the Van Slyke - Neil manometric apparatus and carbon dioxide liberated was collected and measured by the procedure described by Van Slyke and Folch for micro-combustions. Briefly, this procedure is as follows. The gas is drawn over into the chamber of the manometric apparatus and is absorbed in carbon dioxide-free 0·5 *N* sodium hydroxide plus 0·3 *M* hydrazine, the hydrazine serving to reduce any halogens evolved. The unabsorbed gases are then ejected and the carbon dioxide is set free by acidification with 2 *N* lactic acid and measured manometrically.

DETERMINATION OF INORGANIC CARBON IN SOIL

The recognised methods of determining inorganic carbon in soil all depend on measuring the carbon dioxide evolved when the soil sample is treated with dilute hydrochloric acid. If the soil were boiled with acid, decarboxylation and oxidation of some soil organic matter would probably produce carbon dioxide, especially if the soil contained manganese dioxide. It is customary, therefore, to add a reducing agent, such as ferrous chloride, to the hydrochloric acid, and to keep the boiling point as low as possible by carrying out the determination under reduced pressure. The need for these precautions has been emphasised by recent work which has shown that soils contain large amounts of uronide-like material. The technique developed by Van Slyke and Folch for determining organic carbon was found to require no modification for inorganic carbon in soil beyond substituting 2 ml. of an 0·5 *N* solution of hydrochloric acid containing 14 g. of ferrous chloride per litre for 2 ml. of combustion fluid. Decomposition of soil carbonates was carried out under reduced pressure since ejection of air from the combustion tube is the first step in the Van Slyke and Folch procedure. Decarboxylation of uronide material was minimised by limiting the boiling period to 1·5 minutes. The method is rapid, a complete analysis, weighings included, taking only 20 minutes.

DETERMINATION OF ORGANIC CARBON IN SOIL

In any combustion method of determining organic carbon in soil account must be taken of the fact that many soils contain carbonates. This inorganic carbon must either be removed before the determination of organic carbon or be determined separately and subtracted from the total carbon. If a dry combustion method is to be employed it is preferable to remove carbonates beforehand, because high temperatures and a lengthy combustion period (1½ hours) are required to ensure complete decomposition of calcium carbonate. The only reagent suitable for preliminary destruction of carbonates is sulphurous acid, as it is easily removed by evaporation. It is customary to weigh the soil sample into a combustion boat, add sulphurous acid and, after a few hours, remove the excess of acid by heating the boat on a steam-bath or by leaving it overnight in an oven at 105° C. This treatment is repeated until all carbonates have been destroyed. Objections can be raised against the procedure on the following grounds—

(i) Although sulphurous acid has reducing properties which probably minimise the oxidation and decarboxylation of soil organic matter during the acid treatment, some loss of organic carbon may occur on heating, especially if several treatments with sulphurous acid are necessary.

(ii) It is often difficult to decide when all carbonates have been destroyed and the sulphurous acid treatment can be discontinued.

(iii) There is some danger of mechanical loss during the repeated additions of sulphurous acid and evaporations.

The sulphurous acid treatment can be omitted if the soil contains no inorganic carbon, but it is often difficult to decide if this is the case as some soils contain relatively inactive carbonates that are not readily detected in a qualitative test. It is generally better to determine inorganic carbon separately than to run the risk of including it in the organic carbon. As will be shown below, for the wet combustion method of Van Slyke and Folch it is neither necessary nor preferable to remove carbonates from the soil sample before analysis. Organic carbon is determined from total carbon by subtracting inorganic carbon, determined separately.

When determining the carbon contents of certain highly organic soils by the method of Van Slyke and Folch, it was found that mechanical loss of material took place during addition of the soil sample to the combustion tube, owing either to the lightness of the soil or to its tendency to adhere to the spatula during transference to the tube. This difficulty was overcome by weighing the sample in a small porcelain combustion boat and gently sliding the boat with sample into a slightly inclined combustion tube.

DETERMINATION OF ORGANIC CARBON IN SOIL EXTRACTS

The Van Slyke and Folch method of determining organic carbon was found to be applicable to soil extracts. The method was tested on a solution obtained by extracting a non-calcareous Cambridgeshire fen soil with neutral 0·1 *M* sodium pyrophosphate. Samples of this extract were pipetted into combustion tubes and evaporated to dryness before analysis by leaving the tubes overnight in a large evacuated desiccator. If carbonates are present the soil extract is slightly acidified before evaporation by cautious addition of hydrochloric acid from a capillary tube.

RESULTS

INORGANIC CARBON IN SOIL—

The method was tested with the nine soils used in the investigation conducted by the Organic Carbon Committee. The results, expressed as percentages of inorganic carbon in oven-dried soil, are given in Table I, which also shows the mean values and standard deviations of the results obtained by the methods tested by the Committee.

TABLE I

INORGANIC CARBON, %

Soil No.	Previous detmns. by recognised methods: Mean	Previous detmns. by recognised methods: Standard deviation	Present method	Deviation of result by present method from previous mean
1	0·041	0·003	0·038	0·003
2	1·28	0·06	1·28	0·00
3	2·26	0·05	2·26	0·00
4	0·023	0·029	0·020	0·003
5	0·00	—	0·00	—
6	0·00	—	0·00	—
7	0·85	0·05	0·84	0·01
8	0·05	0·01	0·057	0·007
9	0·00	—	0·00	—

With calcium carbonate the maximum error of the method was found to be 1 per cent.

ORGANIC CARBON IN SOIL—

Table II shows the recoveries obtained with the Van Slyke and Folch wet combustion method when calcium carbonate and mixtures of calcium carbonate and soil were analysed. The soil employed contained no inorganic carbon. The amount of organic carbon present in the mixtures was calculated from the weight of soil taken and the organic carbon content of the soil.

TABLE II

TOTAL CARBON

Carbon taken, mg.		
Organic	Inorganic	Carbon found, mg.
—	3·44	3·43
—	2·81	2·81
1·12	2·16	3·27
1·53	1·92	3·44

Table III gives the results of analyses carried out by the Van Slyke and Folch method on the nine soils used in the co-operative study conducted by the Organic Carbon Committee. Soil samples containing from 2 to 3·5 mg. of carbon were taken for analysis and organic carbon figures were obtained from total carbon by subtracting inorganic carbon, which was determined separately. Some of the analyses were later repeated on soil samples containing only 0·3 to 0·7 mg. of carbon; the same results were obtained. Table III also gives the mean values and standard deviations of the results obtained with the dry combustion methods tested by the Organic Carbon Committee. All results are expressed as percentages of organic carbon in oven-dried soil.

TABLE III

ORGANIC CARBON %, FOUND FROM TOTAL MINUS INORGANIC CARBON

Soil No.	Previous detmns. (12) by various dry combustion methods: Mean	Standard deviation	Present method	Deviation of result by present method from previous mean
1	0·53	0·03	0·50	0·03
2	2·91	0·12	2·85	0·06
3	0·80	0·07	0·70	0·10
4	17·83	0·27	17·77	0·06
5	2·72	0·05	2·69	0·03
6	2·74	0·06	2·83	0·09
7	2·36	0·06	2·31	0·05
8	1·86	0·06	1·89	0·03
9	2·07	0·03	2·04	0·03

The organic carbon figures obtained by the Van Slyke and Folch method after treatment of the soils with sulphurous acid are given in Table IV. Four methods of treatment were studied:

(i) The soil sample was weighed into a combustion tube and treated with 5 per cent. sulphurous acid. After a few hours the excess of acid was removed by leaving the tube overnight in an evacuated desiccator.

(ii) As above, but excess of sulphurous acid was removed by placing the tube in a boiling water-bath.

(iii) As in (i), but excess of sulphurous acid was removed by leaving the tube overnight in an oven at 105° C.

(iv) The soil sample was weighed into a small porcelain combustion boat, such as is used for semi-micro dry combustions, and treated with 5 per cent. sulphurous acid. After a few hours the excess of acid was removed by heating the boat on a steam-bath. For determination of organic carbon the boat with sample was slid gently into a combustion tube.

Soil samples containing about 3 mg. of organic carbon were taken for analysis and 2 ml. of 5 per cent. sulphurous acid were used for each treatment. All treatments were repeated until no evolution of carbon dioxide could be observed on further addition of sulphurous acid, and in some instances beyond this point. In Table IV the number of treatments performed is shown in brackets after each result; all results are expressed as percentages of organic carbon in oven-dried soil.

TABLE IV

ORGANIC CARBON %, FOUND AFTER SULPHUROUS ACID TREATMENT

	Methods of sulphurous acid treatment			
Soil No.	(i)	(ii)	(iii)	(iv)
1	0·51 (1)	0·50 (1) 0·49 (2)	0·49 (1) 0·48 (2)	0·51 (1)
2	2·86 (3)	2·71 (3) 2·68 (4)	2·66 (3)	2·85 (3)
3	—	0·69 (4) 0·68 (5)	—	—
4	17·75 (1)	17·0 (1) 16·7 (2)	16·8 (1)	17·2 (2)
5	2·70 (1)	2·61 (1) 2·58 (3)	2·60 (1)	—
6	—	2·73 (2)	—	—
7	—	2·24 (2) 2·23 (3)	2·20 (2)	2·28 (2)
8	1·86 (2)	1·85 (2)	1·80 (3)	1·86 (2)
9	2·03 (1)	1·90 (2)	—	2·02 (1)

ORGANIC CARBON IN SOIL EXTRACTS—

Table V gives the results obtained with the neutral sodium pyrophosphate extract of fen soil. One-ml. samples of this extract were pipetted into porcelain boats of the type used by Rickson in his semi-micro dry combustion apparatus and evaporated to dryness by leaving the boats for a few hours in an evacuated desiccator. The organic carbon contents of the residues in the boats were determined by Rickson's method. Five-ml. samples of the extract were then diluted to 10, 20 and 40 ml., and 1-ml. samples were pipetted from each diluted solution into Van Slyke and Folch combustion tubes and evaporated. Before evaporation one of the samples was acidified with hydrochloric acid and another was treated with 1 ml. of 0·5 *M* sodium carbonate and then acidified. The carbon contents of the samples taken from the diluted extracts were determined by the method of Van Slyke and Folch.

TABLE V

ORGANIC CARBON IN SOIL EXTRACT

Original extract represented by sample taken, ml.	Method of analysis	Carbon found, mg.
1·000	A	4·72
0·500	B	2·36
0·500*	B	2·35
0·500†	B	2·36
0·250	B	1·17
0·125	B	0·59

A Dry combustion method of Rickson.
B Wet combustion method of Van Slyke and Folch.
* Sample acidified with hydrochloric acid before evaporation.
† Sample treated with 1 ml. of 0·5 *M* sodium carbonate and acidified with hydrochloric acid before evaporation.

DISCUSSION

INORGANIC CARBON IN SOIL—

From Table I it can be seen that the method described for inorganic carbon is at least as accurate as established procedures. With the nine soils tested the deviations of the results given by the method from the mean values of the results obtained by the recognised techniques fall well within the standard errors of the latter.

ORGANIC CARBON IN SOIL—

The results given in Tables II and III show that the organic plus inorganic carbon content of a soil is determined by the Van Slyke and Folch wet combustion method and that the organic carbon content can be determined accurately from the total carbon figure by subtracting inorganic carbon, determined separately. Table III shows that, with two exceptions (Soils 3 and 6), the deviations of the results obtained for organic carbon by this method from the mean values of the results previously obtained by dry combustion procedures fall within the standard errors of the latter. For Soil 6 the result given by the Van Slyke and Folch method is in close agreement with the highest values given in the Organic Carbon Committee's report; these values differ from the mean (2·74 per cent.) by 0·1 per cent. carbon. Since this soil contains neither carbonates nor chlorides there should be no special analytical difficulties in the determination of organic carbon. It is therefore suggested that some variation in the sub-samples distributed to the various laboratories participating in the Organic Carbon Committee's investigation may account for the differences between the results reported for this soil. The close agreement of the results obtained with the Rothamsted sample of Soil 6 supports this explanation; Warren[1] in 1935 found 2·84 per cent. of organic carbon by the Ter Meulen dry combustion method, and Rickson[2] obtained 2·85 per cent. in 1947 by a modification of the same method; the Van Slyke and Folch method gave 2·83 per cent. Soil 3 presents special analytical difficulties to dry combustion methods since it contains less than 1 per cent. of organic carbon in presence of about 19 per cent. of calcium carbonate. Complete removal of carbonates from this soil with sulphurous acid before dry combustion is likely to prove difficult, especially if the soil contains unreactive limestone. Soil 3 also contains chlorides, which are known to lead to high results with certain dry combustion methods unless steps are taken to prevent interference. These sources of error are avoided if organic carbon is determined by the Van Slyke and Folch method, since chlorides do not interfere and it is not necessary in this case to remove carbonates.

From a comparison of the results given in Tables III and IV it is evident that treatment of a soil with sulphurous acid can lead to loss of organic carbon, the magnitude of the loss being dependent upon the method used for evaporation of the excess of acid. The loss is negligible when evaporation is carried out at room temperature (Method (i)), but can become serious if heat is used to speed evaporation. In the latter case the magnitude of the loss apparently depends upon the time of contact of the soil with hot acid. Thus it is least in Method (iv), in which evaporation of sulphurous acid is rapid, and greatest in Method (iii), in which evaporation is very slow. Method (iv) is the one generally employed to remove carbonates from calcareous soils before determining organic carbon by dry combustion methods. Although destruction of organic carbon by this method is generally slight, it is not always negligible. Clearly, Method (i), though slower, is preferable, and it is therefore suggested that this method of removing carbonates should be adopted in pretreating calcareous soils before determining organic carbon by dry combustion methods. Since the Van Slyke and Folch method, unlike the dry combustion techniques, requires no inconvenient modification for the determination of organic plus inorganic carbon, it is preferable to avoid the use of sulphurous acid by determining organic carbon from total and inorganic carbon figures.

ORGANIC CARBON IN SOIL EXTRACTS—

The results given in Table V show that:

(i) Micro and sub-micro quantities of organic carbon in soil extracts can be determined accurately by the Van Slyke and Folch method.

(ii) No loss of organic carbon by decarboxylation or other processes takes place if the soil extract is acidified with hydrochloric acid before evaporation *in vacuo*.

(iii) The organic carbon content of a soil extract can be determined accurately by this method even if a large amount of inorganic carbon is present in the extract.

This method of determining organic carbon in soil extracts has proved to be extremely useful in the study of soil organic matter, for it is rapid and can be employed with extracts obtained by treating soil with sodium hydroxide or sodium carbonate solutions, the classical humus extractants. It has patent advantages over the other methods that have been used for the purpose. In these the extract is heated in acid solution with an oxidising agent, such as dichromate or permanganate, and reduction of the oxidising agent by the carbon in the extract is measured by a titration procedure. Various factors, such as strength of acid and duration of boiling, affect the results; correction factors must be applied to all figures obtained. Since the correction factor varies with the soil and can only be determined accurately by dry combustion of a sample of the extract, it is obvious that the methods are, at best, tedious and unreliable.

CONCLUSIONS

The methods described above have several advantages over those at present employed in soil analysis. While as accurate as established techniques they are quicker and simpler and can deal with smaller quantities of material. Beyond the initial cost of the Van Slyke-Neil apparatus the reagents and apparatus employed are inexpensive and only one apparatus is required for all determinations, an important consideration if bench space is limited. The method reported for determination of organic carbon avoids the dangers involved in removal of carbonates from calcareous soils with sulphurous acid and is not affected by the presence of other substances that sometimes cause difficulty in dry combustion methods. The methods described in this paper have merits that commend their adoption for the routine analysis of soils.

SUMMARY

The use of the Van Slyke - Neil manometric apparatus for the determination of organic and inorganic carbon in soil and of organic carbon in soil extracts is described. Organic carbon is determined by the method of Van Slyke and Folch, and inorganic carbon by a modification of the same method. The methods are as accurate as established procedures and can deal with micro quantities of material. They are rapid and simple and seem well suited for the routine analysis of soils.

The author is indebted to Mr. R. G. Warren for helpful criticism and advice.

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The Estimation of Aldehydes, Ketones and Acetals by means of the Hydroxylamine Hydrochloride Method

BY J. G. MALTBY AND G. R. PRIMAVESI

INTRODUCTION

THE first description of this method, depending on the titration of the acid liberated when hydroxylamine hydrochloride forms an oxime by reacting with the carbonyl group of aldehydes or ketones, was given by Brochet and Cambier[1] for estimating formaldehyde, methyl orange being used as indicator. Walther[2] applied the method to the estimation of citral in lemon oil, using an alcoholic solution of the reagent in presence of sodium bicarbonate, heating under a reflux condenser and titrating the experiment and a blank to both phenolphthalein and methyl orange. Bennett[3] modified Walther's method by adding enough alcoholic potash to combine with the liberated acid. Bennett and Donovan[4] extended Bennett's method to formaldehyde, acetone, benzaldehyde and cinnamic aldehyde. For formaldehyde and acetone the reaction was carried out in a stoppered bottle at room temperatures for 2 hours. Bromophenol blue was tried as indicator, but best results were obtained with methyl orange as outside indicator. Camphor gave unsatisfactory results.

Marasco[5] used aqueous reagent and methyl orange as indicator, for acetone and formaldehyde, finding that the reaction under his conditions only went to 94·4 per cent. If the liberated acid is allowed to accumulate the reaction slows down, but in neutral solution 5 minutes are sufficient for the reaction to go to the 94·4 per cent. value. A second portion of neutralised reagent is used as a blank. In the experiment the titration is incremental. Ethyl alcohol was found to have no effect when present in small amounts.

Bennett and Cocking[6] applied the method to carvone in essential oils, using dimethyl yellow as indicator, and heating in stoppered tubes to 75° C. Bryant and Smith[7] added pyridine to displace the equilibrium, but this was found to cause an indistinct end-point by Dermer, Wilson, Johnson and Dermer.[8] Mitchell, Smith and Bryant[9] used the Karl Fischer method to estimate the water formed in the reaction, excess hydroxylamine being first destroyed by the addition of sulphur dioxide and pyridine.

Doering[10] found that with acetaldehyde the reaction rate increased in presence of acid, and therefore used an aqueous acid reagent. Pevtzov[11] used bromophenol blue as indicator, finding that the reaction went to 99·5 per cent. of completion; they used an aqueous reagent for the estimation of acetone.

EXPERIMENTAL

Our first use of the method was in estimating traces of aldehyde (probably butyraldehyde) in commercial butanol made by catalytic hydrogenation of crotonaldehyde. Bromophenol blue was used as indicator, with a 2 per cent. alcoholic solution of the reagent neutralised in bulk. With 25 g. of sample and 25 ml. of reagent the first end-point indicated 0·20 per cent. of butyraldehyde and an incremental titration lasting half an hour 0·35 per cent. When the solution was left for 40 minutes before titrating, the first end-point gave 0·32 per cent., increasing to 0·37 per cent. on further incremental titration over a further $\frac{1}{4}$ hour. By adding an excess of alkali first and titrating back immediately, only 0·22 per cent. was found, increasing on standing to 0·35 per cent. Finally, by allowing to stand 2 hours before titrating, the first end-point showed 0·37 per cent., and this did not increase on standing.

Tests were carried out on 2 per cent. solutions of crotonaldehyde and butyraldehyde in butanol. The aldehydes were 80 to 95 per cent. pure as found by several methods of analysis. The butanol was made as free from aldehyde as possible by treatments with caustic alkali, *m*-phenylenediamine and sodium, and finally contained 0·04 per cent. of aldehyde when tested by the above method. Tests on 10 ml. of the synthetic solutions and 20 ml. of reagent showed about 75 per cent. (allowing for the aldehyde content of the butanol) of the correct figure when the first end-point was used, and 95 per cent. on incremental titration. Repeat tests in which 30 ml. of reagent were used and the solutions left for 1 hour before being titrated showed about 98 per cent. of the correct figure at the first end-point, increasing to 99 or 100 per cent. on incremental titration over 5 to 10 minutes until constant.

A number of tests were then carried out with the butanol that had been freed as far as possible from aldehydes. Some of these tests used an aqueous reagent and an aqueous solution of the butanol, whilst others used an equal volume of 95 per cent. ethyl alcohol (free from aldehydes according to Schiff's test) with the alcoholic reagent. It was found that alcohols themselves apparently cause a shift in the end-point towards the acid side, whilst addition of water causes the end-point to shift towards the alkaline side. The effect is that in the above method an apparent butyraldehyde content of about 0·02 per cent. would be found in aldehyde-free butanol.

Tests were also carried out on the relative suitability of bromophenol blue and methyl orange in presence of solvents. These two indicators cover nearly the same pH range, but behave very differently in presence of solvents, as shown by Kolthoff.[12] The first tests were carried out with acetone as solvent. In anhydrous acetone both indicators will reveal traces of strong mineral acid, but in presence of water methyl orange is much less sensitive than bromophenol blue. Both, however, show the "neutral" indicator colour at the wrong place, *viz.*, methyl orange when a fair amount of acid is present and bromophenol blue when a trace of alkali is present. Increasing the acetone concentration causes the solution apparently to become alkaline to methyl orange, but acid to bromophenol blue, and the reverse occurs on diluting with water. Similar results were obtained with butanol, complicated by the fact that two layers are formed. It was also noticed that, with both solvents, both indicators could give certain colours that could not be matched in aqueous solution at any pH. Bromophenol blue also always gives a peculiar dichroic colour except when showing the fully acid (yellow) colour. Some tests were carried out by the usual method, with alcoholic reagent and dimethyl yellow as indicator, but the latter appeared less satisfactory than bromophenol blue.

The next tests were on the analysis of acetone. An aqueous solution of the sample was used, with an aqueous solution of the reagent containing bromophenol blue, neutralised in bulk. The end-point was matched against a control, in which the volume should equal the final volume in the experiment, as the end-point is much less distinct than in most titrations. The reaction was found to be incomplete if the titration was carried out immediately, possibly owing in part to the volatility of acetone. For this reason it was necessary to use a stoppered flask, shaking and allowing the solution to stand for 15 minutes before titrating. The figure obtained also increased as the excess of reagent was increased, up to a maximum, at which 97 to 98 per cent. of the theoretical figure was obtained. In spite of the relatively poor end-point very good duplication could be obtained. It was later found that the aqueous reagent neutralised in bulk developed acidity on storage; so the alcoholic reagent was afterwards used for most tests. The slight complication introduced by having to keep "solvent" concentrations similar in the experiment and the blank was more than counterbalanced by the greater speed of reaction, by the fact that the reaction apparently goes to completion under the conditions used, and as already mentioned by the fact that the alcoholic reagent neutralised in bulk is stable.

Other methods of estimating aldehydes and ketones are used in certain circumstances, when confirmation of results is required, and where the hydroxylamine method is insufficiently sensitive (*i.e.*, for traces of aldehyde in ethanol) or unsuitable (*i.e.*, for traces of aldehyde in glacial acetic acid).

The method as at present carried out is as follows.

METHOD

REAGENT—

Twenty grams of hydroxylamine hydrochloride are dissolved in 100 ml. of water and the solution is made up to 1 litre with 95 per cent. alcohol. Twenty-five ml. of a 0·2 per cent.

solution of bromophenol blue in alcohol are added and the colour of the solution is adjusted to the neutral dichroic green/red by adding 2 *N* sodium hydroxide or hydrochloric acid (usually alkali is required). Sulphuric acid must not be used as hydroxylamine sulphate is not very soluble in alcohol.

PROCEDURE—

The reagent is added to the sample in a 250-ml. conical flask. Enough sample is taken to require from 10 to 20 ml. of 0·2 *N* alkali in the titration, *i.e.*, corresponding to 0·002 to 0·004 g.-mol. of aldehyde, and the volume of reagent used is at least three times the volume of 0·2 *N* alkali required in the titration. We aim at requiring a titration volume of about 20 ml. and use a standard volume of 70 ml. of reagent. At the same time an equal volume of reagent is taken in another flask to act as a blank and any water or alcohol added with the sample must be added to the blank in equal amount. This is because the colour of the indicator depends on the concentration of both water and alcohol.

The sample flask is now allowed to stand for about 5 minutes and then titrated with 0·2 *N* alkali to match the blank. Before final matching, a volume of water, equal to the titration volume added to the sample, must be added to the blank. Matching is best done by holding the two flasks side by side and viewing horizontally through the liquid at a good strong white light (a white cloud is ideal); in this way the end-point can be accurately judged to within less than one drop. After the two flasks have been matched they should be allowed to stand for a further 5 minutes and compared again. This is because some higher aldehydes and ketones react slowly. When the contents of the sample flask have finally ceased to fade, the total titration gives the free aldehyde or ketone. Sample and blank are then placed on the water-bath with a knob in the top for 20 minutes, after which time they are removed and cooled and the sample is re-titrated to match the blank. This second titration gives acetals and also very slowly-acting aldehydes and ketones which are sometimes not determined in the cold.

The above method is the general method. For special samples, *e.g.*, alcohol containing acetaldehyde and much acetal, modification may be necessary. Lower aldehydes (except formaldehyde, which reacts slowly) react almost instantaneously, and the lower acetals are very easily hydrolysed (again excepting formal, $CH_2(OCH_3)_2$, which is not hydrolysed by neutral reagent even on heating, but is hydrolysed on heating with acidified reagent). Hence to determine acetaldehyde and acetal in presence of each other, it is necessary to titrate in the cold as quickly as possible before heating to determine acetal.

With unknown or untried aldehydes a useful check on the completeness of reaction is to add a further measured quantity, say 10 ml., of reagent to both blank and sample after the first titration is complete. If blank and sample still match, enough reagent was originally added; if not, it is advisable to repeat with less of the sample. Paraldehyde is not acted on in the cold, providing the solution is kept neutral or alkaline. If paraldehyde is known to be present the sample should be added to a reagent made alkaline with a known amount of alkali and a similar amount of alkali added to the blank. The equivalent volume of acid is added to the blank and the sample titrated after standing 20 minutes (reaction is slow in alkaline solution) to match the blank. In this case the difference between the amount of acid added to the blank and that added to the sample is due to aldehyde other than paraldehyde present. Paraldehyde itself cannot be satisfactorily estimated by this method; a special method is necessary.

The method described has been used satisfactorily for formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, acrolein, crotonaldehyde, ethyl propyl acrolein, ethyl hexaldehyde, acetone, methyl ethyl ketone, methyl propyl ketone, methyl *iso*butyl ketone and many other less well known aldehydes and ketones.

In order to get accurate and reproducible results it is essential not to vary this procedure in any way, without good cause and without testing the effect of such variation on a known amount of aldehyde. Special attention must be paid to adding to the blank the same quantities of water or alcohol that are added to the sample in order to keep concentrations equal in both flasks.

Note—Some 95 per cent. alcohol may contain appreciable amounts of acetal; in such circumstances the reagent, after being made up, should be warmed on the water-bath for about half an hour before being finally neutralised; after this treatment the colour should remain stable indefinitely.

PROCEDURE TO BE ADOPTED FOR ACID SAMPLES—

Small amounts of very weak organic acids have no effect on the indicator, but the stronger acids such as acetic, oxalic, citric, etc., have an effect, though not a quantitative one. This effect may be allowed for as follows.

Two samples are taken, one being the normal sample treated as described above; to the other is added a volume, equal to the volume of reagent added to the first sample, of a solution containing 25 cc. of 0·2 per cent. alcoholic bromophenol blue in 100 ml. of water made up to 1 litre with 95 per cent. alcohol (this is the reagent without the hydroxylamine). This second sample, so treated, is then titrated with 0·2 *N* alkali to match the blank and the titration volume obtained is to be subtracted from the hydroxylamine titration volume as a correction for the acidity of the sample.

This modification has been found useful when analysing reaction mixtures obtained by oxidation of various aldehydes.

PROCEDURE TO BE ADOPTED FOR BASIC SAMPLES—

One example of a basic sample is pyridinised industrial methylated spirit. The sample is neutralised to bromophenol blue by adding dilute sulphuric acid, and an equal quantity of bromophenol blue is added to the blank so that the final colours will match correctly.

A special modification of the main method, in which water is used in the control test, was worked out as a limit test for aldehydes in ethyl alcohol for the British Standard Specification No. 507 (1933). This method cannot be used for the estimation of aldehydes in spirit, unless the blank of about 0·013 per cent. w/v of aldehydes is subtracted from the figure obtained.

Tests on 1 per cent. solutions of acetaldehyde in alcohol showed that in a freshly made solution all the aldehyde reacted in the cold. After keeping several months, however, only one-fifth of the aldehyde reacted in the cold, the other four-fifths being present as acetal, reacting after warming and cooling. It seems likely that in old samples of butanol the butyraldehyde may be present as butyral, and that the necessity for an incremental titration is due more to this than to slow reaction of butyraldehyde itself.

For the estimation of traces of acetone in *iso*propanol, a modification was worked out to avoid the use of aldehyde-free 95 per cent. ethyl alcohol in the control experiment. In this, 25 ml. of sample were mixed with 0·6 ml. of 0·2 per cent. bromophenol blue solution, the mixture was neutralised to the green colour, and only 5 ml. of alcoholic reagent were added. This solution was then titrated to match a control of 30 ml. of reagent. On a synthetic solution containing 0·10 per cent. of acetone, this method showed 0·096 per cent., whereas our usual method showed 0·095 per cent. On a sample of *iso*propanol the figures found were respectively 0·014 and 0·012 per cent. by the two methods. In our usual method it was found rather difficult to match the colours because of the large volume of sample that had to be taken, and better matching was obtained by adding extra indicator to both experiment and control, the figure then being found to be 0·015 per cent. of acetone. With this sample another laboratory modified the method, using bromocresol green as indicator, and matching against the neutralised reagent itself. Owing to the pH change being at the wrong place this modification gave high results. With the synthetic mixture containing 0·10 per cent. of acetone, figures of 0·16 and 0·18 per cent. were found, and with the sample in question the figures were found to be 0·046 and 0·105 per cent., the latter figure being obtained when more bromocresol green was added to the experiment.

Our method has been compared with the routine method used in another laboratory. This method uses normal aqueous hydroxylamine sulphate reagent, neutralised just before use, with methyl orange/xylene cyanol screened indicator. Normal alkali is used in the titration, and usually no control is used. Although the use of normal alkali helps to give a sharper end-point, we consider that the accuracy of the figure obtained is not as good as by our method.

It is possible to distinguish between, and estimate separately, substances that react rapidly with hydroxylamine in the cold and those that react slowly. This is done by continually matching the sample against the blank by an incremental titration. The total titration is plotted against the time. When the increase due to the slowly-acting substance becomes steady, the slope may be extrapolated back to zero time, giving the titration due to the rapidly-acting substance. Heating and cooling until no further increase takes place will then complete the reaction of the slow reactor. In this way methyl *iso*butyl ketone and di *iso*butyl ketone were estimated separately in a mixture.

SUMMARY

A description is given of a satisfactory technique for the hydroxylamine hydrochloride method of estimating aldehydes, ketones and acetals, using alcoholic reagent. Precautions and modifications found necessary when dealing with certain carbonyl compounds and mixtures are given.

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The Determination of Chromium in Cast Iron and Steel

BY T. S. HARRISON AND H. STORR

AN absorptiometric method for the determination of chromium in steel was developed recently by De Lippa,[1] who used potassium bromate as oxidising agent. The main advantages of his procedure were (1) avoidance of the use of silver nitrate (as in the persulphate method) and (2) selective oxidation of Cr^{III} to Cr^{IV} with oxidation of the manganese only to the quadrivalent state, not to permanganic acid. Speed and accuracy were claimed provided that a number of precautions were taken. The procedure could be incorporated in Vaughan's composite scheme[2] if desired.

The object of this short research was to apply this method, with appropriate modifications, to the assay of chromium in graphitic cast iron and other ferrous alloys received in our laboratories.

EXPERIMENTAL

As composite schemes are not in general use in this laboratory, it was decided to take 1 g. of sample through the procedure, *i.e.*, omitting the fractionation after dissolution. This necessitated increases in the amounts of reagents subsequently added and a consequent change in the absorptiometric conditions. Filtration was introduced to remove graphite. The precautions laid down were necessary and on no account must the liquid boil down to low bulk during the oxidation. Readings taken at intervals showed the dichromate colour to be stable for several hours.

Following a number of determinations it was decided to check the results by titration. The contents of the cells were poured back, each solution was diluted to about 250 ml., an excess of 0·1 N ferrous ammonium sulphate was added and 0·1 N potassium permanganate solution was run in to give a pink colour stable for at least 2 minutes. The end-point was very clear and definite and agreement was very good. This dual ending is a distinct advantage, especially when a result is in doubt or the chromium content exceeds the ranges of available graphs.

PROCEDURE—

Graphitic irons—Dissolve 1 g. of sample in 40 ml. of Spekker acid, add a little water, oxidise dropwise with concentrated nitric acid and boil off nitrous fumes. Filter through a

Whatman No. 41 paper and wash with hot water. The volume of the filtrate and washings should be about 80 to 90 ml. Heat just to boiling on the hot plate, add 20 ml. 6 per cent. potassium bromate solution and boil gently for 5 minutes. Introduce 10 ml. of 20 per cent. ammonium sulphate solution and boil for 10 minutes. Add 8 ml. of 20 per cent. hydrochloric acid and boil gently for a further 10 minutes, replacing any evaporation losses with hot distilled water. Cool to below 19° C., dilute to 120 ml. with water, fill a 2-cm. or 4-cm. cell and measure the absorption with Ilford Spectrum Violet filters No. 601 and setting water-to-water 1·2. Convert to percentage of chromium from a graph (Fig. 1) constructed by using 1-g. quantities of a plain cast iron with increasing amounts of standard potassium dichromate solution (2·8284 g. per litre; 1 ml. ≡ 0·001 g. Cr.).

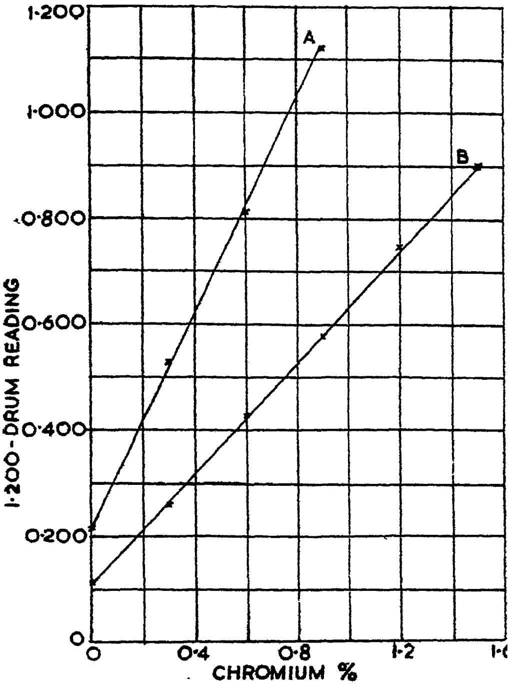

Fig. 1. Chromium in cast iron
Ilford spectrum violet filter No. 601 with Chance No. H 503
A 4 cm. cells B 2 cm. cells
Setting water-to-water 1.2

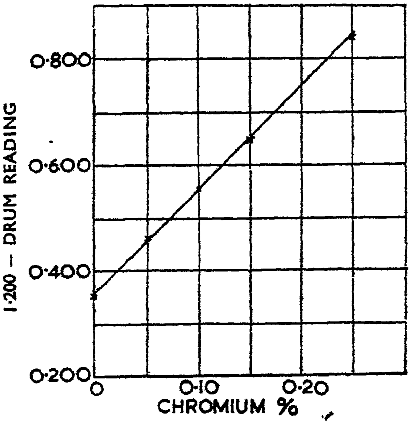

Fig. 2. Chromium in pig iron
Ilford spectrum violet filter No. 601 with Chance No. H503
4 cm. cells Setting water-to-water 1.2

Empty the contents of the cell into the beaker containing the main solution, rinsing with distilled water, and dilute to about 250 ml. Add an excess of 0·1 N ferrous ammonium sulphate solution and titrate back with 0·1 N potassium permanganate solution. 1 ml. of 0·1 N ferrous ammonium sulphate solution ≡ 0·001734 g. of chromium.

Alloys of higher chromium content such as ferrochrome, 30 per cent. chrome cast iron and stainless steel were analysed successfully, the titration ending only being used; 0·3468 g. of ferrochrome or 30 per cent. chrome iron and 0·6936 g. of stainless steel were convenient amounts to take. The amounts of bromate, sulphate and hydrochloric acid and the respective boiling times were increased by 50 per cent. and the solution was diluted to about 400 ml. before titration.

De Lippa's method has also been adapted to the determination of small amounts of chromium in pig iron. For this the Vignal and persulphate oxidation processes were unreliable and the diphenylcarbazide method too long for routine use. Two grams of sample were treated as above and readings taken in 4-cm. cells. The graph used is shown in Fig. 2. The above titration ending was again applicable owing to the very definite end-point.

RESULTS—

The following are typical figures. All are percentages of chromium.

(*a*) B.C.S. STANDARDS—

Description	Certificate figure	Chromium found (Spekker)
Ni–Cr–Mo C.I. "K" (No. 172)	0·41	0·40
Ni–Cr–Mo Steel "B" (No. 189)	0·68	0·70
Cr–V Steel "V" (No. 165)	0·86	0·88
Ni–Cr–Mo Steel (No. 225)	1·04	1·06

(*b*) FOUNDRY CAST IRONS—

(i) Grey cast irons containing up to about 2 per cent. of chromium, but no other alloying elements.

Vignal or persulphate volumetric method	Spekker	Volumetric ending following Spekker reading
1·03	1·02	—
1·12	1·13	—
1·30	1·30	—
1·51	1·55	—
1·75	1·76	—
—	0·50	0·47
—	0·72	0·75
—	0·97	0·97
—	1·13	1·13
—	1·19	1·18
—	1·24	1·27
—	1·32	1·32
—	1·46	1·47
—	1·58	1·56
—	1·74	1·74
1·04	1·01	1·03
1·08	1·09	1·04
1·22	1·20	1·20
1·26	1·27	1·32
1·45	1·41	1·37

(ii) 30 per cent. Chrome iron.

Persulphate, volumetric	Bromate, volumetric
30·1	30·1
30·3	30·2
31·0	31·0
33·8	34·0

(*c*) B.C.S. STANDARDS—

Description	Certificate figure	Chromium found bromate, volumetric
Stainless Steel No. 209	37·42	37·2
High carbon Ferro-chrome No. 204	69·0	68·8

(*d*) PIG IRONS—

Spekker	Volumetric ending following Spekker reading
0·22	0·23
0·22	0·23
0·23	0·21
0·25	0·23

SUMMARY

The bromate oxidation process in modified forms has been applied to the determination of chromium in a variety of ferrous alloys. The analyses may also be completed volumetrically, the end-point being very definite.

The authors thank the Directors of Messrs. Newton, Chambers & Co., Ltd., for permission to publish this work.

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NEWTON, CHAMBERS & CO., LTD.
THORNCLIFFE
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January, 1949

The Determination of Zirconium in Minerals and Refractories by the Tannin Method

BY H. HOLNESS AND R. W. KEAR

THE standard methods for the analysis of zirconium minerals and refractories are all subject to a number of inherent difficulties. This work has been undertaken in an attempt to devise a method in which some of these difficulties are overcome.

In previous papers the precipitation by tannin of both zirconium[1] and titanium[2] from acid chloride solution has been studied and shown to be quantitative for a definite range of acidity. Clean separations of both these elements from substantial amounts of iron, aluminium, vanadium, thorium and the rare earths were achieved. Germanium[3] and tin[4] can also be precipitated under the same conditions, but are not normally present in a zirconium analysis; they can, however, be removed by precipitation of their sulphides, or the germanium by distillation of its chloride. Titanium is the one element that interferes with the tannin precipitation of zirconium, but it interferes also with zirconium analysis by other processes. The zirconium tannin complex includes any titanium present and this must be determined and deducted. In the cupferron method the zirconium precipitate contains the whole of the titanium and minor quantities of thorium and rare earths. The precipitate obtained by the arsenate method must be tested colorimetrically for titanium. The selenite precipitate includes any thorium present and a colorimetric test for titanium is advised. The phosphate method is the only one that yields a zirconium precipitate quite free from titanium, but it is unsuitable for substantial quantities, and the ignited precipitate is not of constant composition. On ignition the cupferron, arsenate and selenite precipitates give off poisonous fumes.

The standard cupferron procedure[5] for the analysis of a zircon mineral or refractory involves two fusions, one alkaline and one acid, double ammonia precipitation and the evaporation of a considerable bulk of liquid followed by the destruction of a large amount of organic matter before the analysis is completed; these time-consuming operations are eliminated or reduced to a minimum in the method described below.

The bisulphate fusion is avoided by dissolving the zirconia in the concentrated sulphuric acid used to dehydrate the silica, and then, after double ammonia precipitation and solution in hydrochloric acid—a step necessary to remove all traces of sulphate—the further analysis is neatly resolved by the use of tannin. The zirconium and the titanium are precipitated together from acid solution, and the iron and aluminium are recovered together from the filtrate when it is made ammoniacal. The final step, the analysis of the tannin precipitates, follows standard practice.

EXPERIMENTAL

The procedure described below was applied to four different zirconium materials, *viz.*, a zircon firebrick, the Brazilian zirconia rock often called baddeleyite, zircon sand from the Travancore deposits, and a sample of technical zirconium oxide. Each was analysed by the cupferron method and found to contain, in addition to zirconia and silica, minor amounts of titania, alumina and ferric oxide.

PREPARATION OF SAMPLE—

The material was prepared by the standard methods used in ore and mineral analysis. As an additional precaution the slimings were made to pass through a G.1 sintered glass filter before they were evaporated to dryness.

PROCEDURE—

About 0·2 to 0·3 g. of sample was mixed with 3 to 5 g. of A.R. sodium carbonate in a covered platinum crucible. Before fusing, a small bunsen flame was applied for a few minutes to remove moisture, then a low-flamed Meker burner was substituted and the blast increased over a period of 10 minutes to the maximum. Fusion was continued for 1 hour. Two methods were used for the removal of the melt from the crucible.

(*a*) The crucible and cover were, when cold, placed in a tall 400-ml. lipless beaker and covered, and then, while the cover was pushed a little to one side, a solution of 20 ml. of concentrated sulphuric acid in 100 ml. of water was poured slowly down the side and the cover replaced. After the reaction had subsided the whole was boiled to expel carbon dioxide. The crucible and lid were then extracted and well washed with water.

(*b*) On completion of the fusion the crucible was allowed to cool slightly (to allow any drops on the lid to solidify), the lid was removed and a piece of platinum wire with a coiled end immersed in the liquid melt. After this had cooled and hardened, a Meker burner with a full blast was placed underneath, and while the crucible was held in a pair of platinum-tipped tongs, tension was applied to the wire until the melt came away cleanly. The Meker was removed and the crucible and melt allowed to cool. The melt was placed in a covered 400-ml. lipless beaker containing 90 ml. of 20 per cent. sulphuric acid and the platinum wire removed and rinsed on completion of the reaction. The crucible and lid were boiled out twice with water and a third time with 10 ml. of 20 per cent. sulphuric acid, the washings and acid being transferred to the beaker.

After solution of the melt had been effected by either one of these methods, (*a*) or (*b*), it was allowed to evaporate in the uncovered beaker over a low flame until fuming occurred. The beaker was then covered and boiled vigorously for 20 to 30 minutes to dissolve the zirconia.

After being allowed to cool it was diluted with water to about 100 ml. and boiled. The silica was filtered on an 11-cm. No. 41 Whatman paper and washed well with hot water and the filtrate, "A," reserved. The precipitate was ashed in a platinum crucible and the silica determined by treatment with hydrogen fluoride. The small residue remaining in the platinum crucible was fused with a little potassium hydrogen sulphate and leached with water into a 100-ml. beaker. A few drops of dilute hydrochloric acid were then added and the solution saturated with hydrogen sulphide; paper pulp was added and the small precipitate of platinum sulphide filtered, washed and rejected. The filtrate was boiled to remove hydrogen sulphide, oxidised with a little concentrated nitric acid, again boiled and then added to the filtrate "A."

Five grams of ammonium chloride were added and 0·88 A.R. ammonia solution (filtered free from silica) was gradually stirred in until the whole was just alkaline to methyl orange. The precipitate was filtered on a 12·5-cm. No. 41 paper with the aid of a little paper pulp, and washed with a cold 2 per cent. solution of ammonium chloride; the filtrate and washings were discarded. The precipitate was dissolved in 50 ml. of diluted hydrochloric acid (1 + 1), boiled, cooled and reprecipitated with ammonium chloride and ammonia. The precipitate was filtered and washed until free from sulphate.

The precipitate was again dissolved in 50 ml. of diluted hydrochloric acid (1 + 1), and the solution boiled and diluted to 200 ml., 5 g. of ammonium chloride were added and the solution boiled, and then a filtered solution containing 1 g. of tannin dissolved in 20 ml. of boiling water was added. To the gently boiling solution about 100 ml. of 2 *N*, filtered, A.R. ammonia solution were slowly stirred in until the liquid was only faintly acid to methyl orange. The whole was allowed to digest and flocculate, a little pulp was introduced and the precipitate was filtered on a 12·5-cm. No. 41 Whatman paper to give the first tannin precipitate (TP^1). This was washed with a hot 2 per cent. solution of ammonium chloride, ashed and weighed; it contained all the zirconia and some titania. The filtrate and washings were boiled, 1 g. of tannin (dissolved as before) was added and 2 *N* filtered A.R. ammonia solution drop by drop until the solution was just not neutral to methyl orange. The liquid was digested and filtered on a 9 cm. paper to give a minor tannin precipitate (TP^2), which was washed as before and ashed to give the remaining titania. With experience it was found that TP^2 could be included in TP^1 by making use of the dark iron tannin complex as an indicator. The ammonia was added until the darkening was just *not* permanent.

The filtrate was boiled and 20 ml. of 2 *N* filtered A.R. ammonia solution added, and the precipitate allowed to digest before being filtered on an 11-cm. No. 41 paper. It was washed with a hot 2 per cent. solution of ammonium chloride to give the final tannin precipitate (TP^3) and contained all the iron and aluminium; it was ashed and the filtrate discarded.

The ashed precipitates TP^1 and TP^2 were combined and fused with potassium hydrogen sulphate. The titania was determined either colorimetrically, or by tannin precipitation from oxalate solution, according to established practice, and the zirconia was obtained by difference.

The combined precipitate of iron and aluminium, TP^3, was also fused with bisulphate. The iron was determined colorimetrically, or by precipitation as ferrous sulphide, and the alumina obtained by difference.

It was sometimes found necessary to correct the alumina figure for a small amount of silica picked up from the glassware while the solutions were ammoniacal, *e.g.*, during the double ammonia precipitation. The presence of this silica became evident on leaching the bisulphate melt of TP^3, and when found it was filtered, ashed, and weighed to provide the necessary correction.

RESULTS

Material analysed	Method of analysis	Loss on ignition %	SiO_2 %	$ZrO_2 + TiO_2$ %	$Al_2O_3 + Fe_2O_3$ %	Total %
Zircon firebrick	Cupferron	0·13	29·49	65·53	4·84	99·99
	Cupferron	0·13	29·40	65·89	4·67	100·09
	Tannin	0·13	29·42	65·80	4·68	100·03
	Tannin	0·13	29·49	65·62	4·94	100·18
Zirconia rock	Cupferron	—	14·31	82·77	3·11	100·19
"Baddeleyite"	Tannin	—	14·27	82·60	3·25	100·12
(Brazil)	Tannin	—	14·26	82·90	2·82	99·98
Zircon sand	Cupferron	0·10	31·61	66·16	2·28	100·15
(Travancore)	Tannin	0·10	31·39	66·37	2·34	100·20
	Tannin	0·10	31·54	66·26	2·26	100·16
Zirconium oxide	Cupferron	0·70	7·90	89·25	2·29	100·14
(technical)	Tannin	0·70	8·10	89·45	2·15	100·40
	Tannin	0·70	7·93	89·30	2·21	100·14

The procedure described above for the determination of silica possesses two commendable features, since

(*a*) there is no danger of alkaline solutions coming into contact with the glass vessels and funnels, and

(*b*) there is no liability of loss by transference of material until the silica is filtered.

In this work we have everywhere prescribed the use of filtered solutions of ammonia in order to reduce to a minimum the introduction of silica into the analysis. It is well known that dilute solutions of ammonia attack glass vessels and it has recently been shown that tannin precipitates silica from slightly ammoniacal solution,[6] hence any extraneous silica increases the amount of TP^3. In the cupferron procedure the presence of this silica becomes apparent during the destruction of the organic matter by fuming with nitric and sulphuric acids and it can be removed before the determination of the aluminium. The presence of this hazard seems to have escaped mention in most of the published procedures on this type of work.

SUMMARY.

A modification of the standard method for determining silica in zirconium materials is described, together with a procedure for the use of tannin in the analysis of zirconium minerals and refractories.

The use of the reagent offers a comparatively rapid method for determining $ZrO_2 + TiO_2$ and $Fe_2O_3 + Al_2O_3$ in these materials.

Attention is drawn to the introduction of silica into the analysis by attack on the glassware by dilute solutions of ammonia.

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January, 1949

The Determination of Butter Fat, Coconut Oil and Palm Kernel Oil in Margarines

By K. A. WILLIAMS

(*Read at the meeting of the Society on Wednesday, October 6th,* 1948)

BOLTON, Richmond and Revis[1] recorded the results of a large number of analyses of fats containing various percentages of butter fat, coconut oil and palm kernel oil. From these they developed two methods, one graphical and one depending on empirical formulae, for

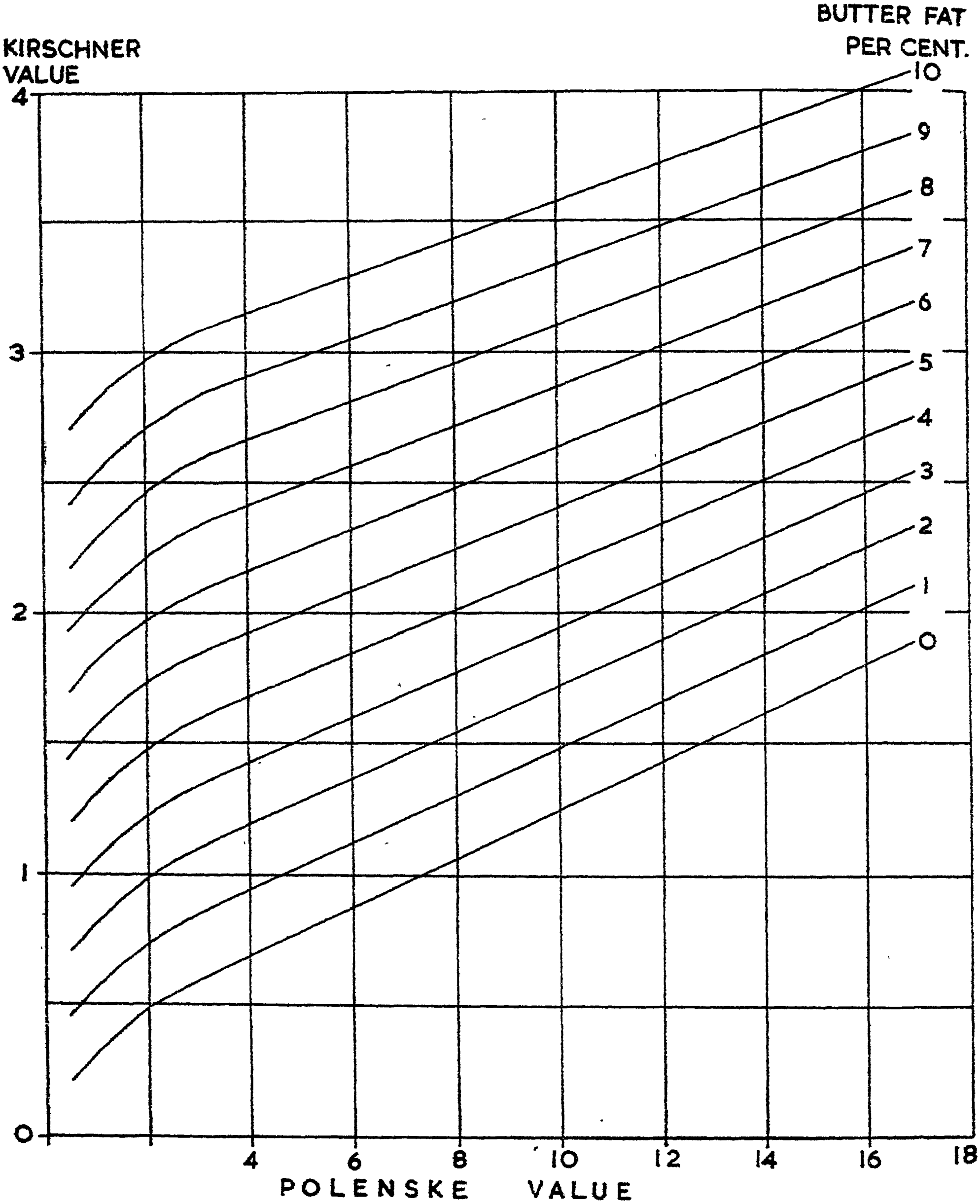

Fig. 1

calculating the percentages of these fats in a mixture from the Reichert, Polenske and Kirschner values.

Both methods have stood the test of time, but the graphical one has proved somewhat cumbersome and not particularly easy to understand. The following procedure will be found simpler in practice; it is based on the original data of Bolton, Richmond and Revis.

The Reichert, Polenske and Kirschner values of the sample having been determined, refer the Polenske and Kirschner values to Fig. 1, and read off directly from this graph the percentage of butter fat in the mixture. Subtract 0·3 from the Reichert value for *each* 1 *per cent.* of butter fat so found, and refer the corrected Reichert value and the Polenske value to Fig. 2.

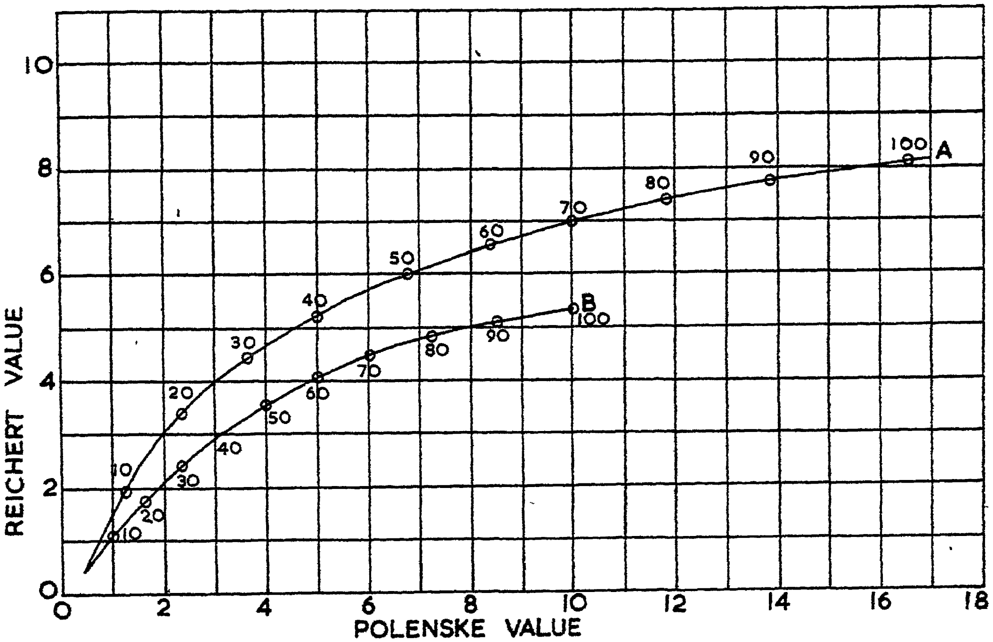

Fig. 2. Curve A.—Coconut oil. Curve B.—Palm kernel oil
Percentage of each oil corresponding to different Reichert and Polenski values

If either coconut oil or palm kernel oil is present, the point so plotted should lie reasonably closely to one or other of the two lines of the graph; then there is a clear indication of which oil is present, and the graph will show the approximate percentage. Mixtures of the two oils lead to points lying between the two lines, and the position of the points depends on the relative proportions of coconut and palm kernel oils present. Fig. 2 should not be applied if oils of the *Palmae* other than coconut or palm kernel oil are present.

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DISCUSSION

MR. J. KING asked if Dr. Williams would kindly state what was the precise technique for carrying out the Reichert - Polenske determination adopted in the late E. R. Bolton's laboratory in compiling the original curves, of which the present curves appeared to be a much simpler version, as any significant deviation would render them far less useful. The method of B.S. No. 769 of 1938, which attains homogeneity in the aqueous phase by four or five double inversions of the 110-ml. distillate, although satisfactory for butter fat, may lead to variable results in equal mixtures of butter fat with coconut or palm kernel fats. The method suggested by the French representative in the International Commission for the Study of Fats, which requires complete homogenisation of oil and aqueous phases appears to be even more objectionable. Presumably Bolton's directions in "Fatty Foods" was adopted, *viz.*, to allow the flask containing the distillate to stand in a water-bath at 10° to 15° C. for 15 minutes, as no doubt homogeneity of the aqueous phase is attained here or at the subsequent filtration with little disturbance of the Polenske acids.

Dr. Williams, in reply, said that the S.P.A. method was drafted by a committee which included the late E. R. Bolton and which discussed his technique in detail with him in his own laboratory. It had also the advantage of having before it much of the original apparatus used by Bolton and his collaborators in putting together the tests of Reichert, Polenske and Kirschner to form the composite whole now called the Reichert - Polenske - Kirschner test. Some of the original apparatus was still in fact in existence in the author's own laboratory. He believed that the S.P.A. method agreed closely with the original technique of Bolton, Richmond and Revis; it certainly agreed with the technique taught to him by Bolton. The British Standards Institution had been careful, in formulating B.S. No. 769, to adhere strictly to the details of the S.P.A. standard method.

He agreed with Mr. King that the fullest possible description of the test was absolutely necessary if different operators were to obtain substantially the same results. The method recently circulated by the International Commission was not nearly sufficiently detailed. Particular attention had been directed by the S.P.A. Committee, after the publication of its report, to the problem mentioned by Mr. King. This difficulty arose from a necessity to homogenise the aqueous portion of the Reichert distillate, without dispersing the insoluble fatty acids floating in it. If the receiving flask is shaken after it has been cooled and just before filtering, there is a probability that liquid insoluble fatty acids will become dispersed and will pass through the filter. The S.P.A. Committee has tried many methods of preventing this accumulation of some liquid insoluble acids in the aqueous phase, but has not found any method offering complete success, especially if the test is carried out on mixtures containing a large percentage of coconut oil. In such cases it is best, at present, to omit the mixing of the contents of the receiver. That is to say, the flask and contents should be cooled as described in the S.P.A. text, and the contents then filtered through the dry, specified filter paper. The liquid should be poured on to the filter in such a way that aqueous liquid reaches it first, the globule of fatty acid following later. In this way it is generally possible to avoid direct contact of insoluble fatty acid with the dry paper, and the amount of insoluble acids contaminating the aqueous phase in the filtrate is kept at a minimum. His experience was that, if as much as possible of the aqueous phase was filtered and then mixed, its composition was very close to that of the whole aqueous phase distilled.

Note added by the author, September, 1949—The French text referred to by Mr. King had been used by analysts in a number of European countries on a sample circulated by the International Commission for the Study of Fats. The results of these tests were discussed at the recent meeting of the Commission in Amsterdam, when it was immediately apparent that the above criticism of the text was justified. As a result of this discussion, further samples are to be examined by members of the Commission, who will use and compare the English and Dutch formulations of the method.

Notes

SUGGESTED MECHANISM OF POISONING BY LIQUID TETRACHLOROETHANE

Few cases of poisoning by the drinking of liquid tetrachloroethane (acetylene tetrachloride) have been recorded. Elliott[1] reports a case where a man died approximately 12 hours after drinking "a small quantity" of the liquid; he appears to have been in a deep coma for the whole of the period. Hepple[2] gives details of a case where the victim went into a coma within 1 hour of drinking the liquid and died 8 hours later; 12 ml. (20 g.) were recovered from the stomach contents.

In one such case where this laboratory performed the toxicological work, 25 g. of liquid tetrachloroethane was recovered by steam distillation from the stomach contents and intestines, and identified by boiling-point and chlorine determinations. The history of the case is very similar to that of those reported. Coma occurred shortly after the taking of the liquid (exact time unknown, but certainly less than 1 hour) and death followed about 9 hours later without consciousness being regained.

The pathological report states that the stomach contents were a dark brown liquid material with a pronounced odour resembling that of chloroform. Moderate congestion of the stomach lining with slight congestion of the liver was also noted.

Lehmann and his associates[3] have shown that the vapour of tetrachloroethane has a toxic action similar to that of chloroform but about four times as potent. Of liquid chloroform,[4,5,6,7] 1 to 2 oz. (28 to 56 g.) is generally considered a minimum lethal dose, although recoveries have followed larger doses.

In view of the high toxicity of tetrachloroethane as compared to chloroform and related chlorinated hydrocarbons, it was decided to find if any metabolic products could be isolated. Examination of the urine (200 ml.) showed it to be very acid in reaction and from it was isolated 0·14 g. of free oxalic acid, although none could be detected in the stomach contents. It would appear probable that this is formed from the tetrachloroethane by hydrolysis followed by oxidation and its disturbance of the calcium metabolism would in part account for the high toxicity of tetrachloroethane.

The writer is indebted to Dr. C. N. Partington, Assistant County Pathologist, Dorset County Council, for permission to quote from his post-mortem report, also to Mr. H. Campbell for assistance with the experimental work and to Mr. E. B. Parkes, M.Sc., F.R.I.C., Director of this laboratory for permission to publish.

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A RAPID METHOD OF ESTIMATION OF AMMONIA NITROGEN IN SEWAGES AND EFFLUENTS

AMMONIA nitrogen in sewage and sewage effluent is normally estimated either by distillation or by direct nesslerisation after some form of preparation of the sample. There is a divergence of opinion as to which is the more suitable or accurate method to use for sewage liquors. Distillation is the choice of the Ministry of Health,[1] which states that "direct nesslerisation . . . cannot be recommended for quantitative purposes but may be found useful for giving an approximate result quickly." On the other hand, the American Public Health Association[2] prefers direct nesslerisation, after a preliminary clarification treatment at pH 10·5, as it avoids errors from hydrolysis of urea and other organic compounds. Even so, distillation by the American method is under closer control than the British approved method since use is made of a phosphate buffer solution which maintains a constant pH of approximately 7·4 during the process.

Apart from the need for accuracy, other factors influencing the choice of method may have to be taken into consideration. For instance, where large batches of routine samples are involved, a direct method can save time, apparatus, and bench space and can be operated in a laboratory where there is no gas supply.

A simple, rapid, direct nesslerisation method applicable to sewages and effluents has been developed in this laboratory. Results with it have been confirmed in the laboratories of the Northern Outfall Works and at County Hall, Westminster Bridge, and are in close agreement with those obtained by the American controlled distillation method. Besides effecting economy of time, space, and materials, it reduces previous sources of error arising from the preparation of samples for direct nesslerisation and is thought to be as accurate as any method hitherto described.

EXPERIMENTAL

Influence of sodium hydroxide on the Nessler colour—Normally, to prepare samples for direct nesslerisation, sodium hydroxide is used to remove substances which otherwise would give a turbidity with Nessler solution. A study of the effect of the concentration of sodium hydroxide in the final matching solution on the Nessler colour complex showed that the yellow colour deepened progressively with increasing of concentration of sodium hydroxide, and that unless the standard contained the same alkali concentration as the prepared sample, serious error could arise.

This effect is shown in Table I, relating to 100-ml. quantities of ammonium chloride solution (1 ml. ≡ 0·000001 g. of nitrogen) containing various concentrations of sodium hydroxide matched against a standard of the same concentration of ammonium chloride containing no sodium hydroxide. Results are expressed as per cent. increase in depth of colour due to the sodium hydroxide.

TABLE I

Concentration of NaOH, g./100 ml.	Increase in depth of colour per cent.	Concentration of NaOH, g./100 ml.	Increase in depth of colour per cent.
0·008	0·0	0·16	6·5
0·016	1·0	0·20	9·5
0·024	1·0	0·32	13·5
0·032	2·0	0·40	16·5
0·040	2·0	0·60	30·0 (*a*)
0·060	4·5	0·72	50·0 (*b*)

All solutions were optically clear except (*a*), hazy, and (*b*), cloudy. Solutions containing more than 0·5 g. of sodium hydroxide per 100 ml. were useless for matching, owing to the turbidity produced.

Avoidance of sodium hydroxide treatment—In testing large batches of samples it is more convenient to use one standard, if possible, than numbers of standards treated with sodium hydroxide to correspond with the concentrations present in the prepared samples. Attention was therefore given to reducing the caustic soda concentration to a minimum, common for all samples. The work of Gledhill and McCanlis[3] had shown that sodium hexametaphosphate (Calgon) solution prevented the development of cloudiness in chalk well waters with Nessler solution. This reagent was therefore tried, and it was found that a small quantity of Calgon solution not only suppressed all cloudiness and haze in the matching solutions, but it eliminated the need for any sodium hydroxide whatsoever to prepare the samples and thereby rendered the intermediate filtration stage unnecessary. All samples of sewages and effluents treated by this Calgon technique have given solutions optically clear. (This does not apply to saline river water samples.) The presence of Calgon solution had no significant effect on the maximum depth of colour produced (unlike sodium potassium tartrate, which also gives optically clear solutions with sewages and effluents prepared without treatment with sodium hydroxide but which deepens the matching colour considerably).

PROCEDURE

Pipette 2 ml. of the filtered sewage or effluent into a 100-ml. measuring flask, partially dilute with ammonia-free distilled water, add 1 ml. of 10 per cent. Calgon solution and complete the dilution to the 100-ml. mark. Add 2 ml. of Nessler solution, allow to stand for 15 minutes and then match against a standard made up by diluting 10 ml. of ammonium chloride solution (1 ml. = 0·00001 g. of nitrogen) and 1 ml. of 10 per cent. Calgon solution up to 100 ml. (*Note*—In our experience optically clear solutions are obtained even with only 0·2 ml. of 10 per cent. Calgon solution for 2 ml. of sewage.)

Comparison of methods—Table II shows ammonia nitrogen contents of sewages and effluents prepared in this manner compared with results obtained by distillation using phosphate buffer solution according to the American Public Health standard method. The solutions were matched in a Klett colorimeter at the Northern Outfall and by a photo-electric colorimeter at the Southern Outfall. The samples tested were average daily sewage and effluent samples; all results are expressed as ammonia nitrogen in parts per million.

TABLE II

	Sewage		Effluent	
	Calgon method	Distillation	Calgon method	Distillation
Southern Outfall 1	39·8	39·4	39·8	38·8
2	39·5	40·2	37·0	36·5
3	43·0	43·0	46·0	45·5
4	45·0	45·0	43·3	42·8
5	39·3	40·5	42·0	42·5
6	37·8	39·3	38·0	39·3
7	47·0	47·7	45·5	46·5
7 (*a*)		46·5		44·5
8	40·3	41·0		
8 (*a*)		41·0		
8 (*b*)	51·5	51·0		
8 (*c*)		50·5		
Northern Outfall 9	30·4	30·0	30·8	29·6
10	31·0	30·5	33·1	32·7
11	27·8	26·7	29·4	28·6
12	31·3	29·6	31·7	29·4
13	28·8	27·8	31·5	29·8

Nos. 7 (*a*), 8 (*a*) and 8 (*c*) were duplicate samples of Nos. 7, 8 and 8 (*b*), but matched against standards not containing Calgon solution.

Nos. 8 (*b*) and 8 (*c*) were sewage of samples No. 8 to which ammonium chloride had been added equivalent to an additional 10 p.p.m. of ammonia nitrogen.

The experiments indicate a close agreement between the two methods, practically within experimental error. It will be seen that added known quantities of ammonium salts have been wholly recovered. The process has been adopted for routine samples in the Southern Outfall laboratory and has given uniformly satisfactory results with a great saving of time.

SALINE RIVER WATERS—

It has been noted above, parenthetically, that this technique is not suitable for tidal river water samples. The salts in such river waters continue to give turbidities even in presence of large quantities of Calgon solution and some form of treatment with caustic soda solution is necessary to obtain clear solutions for matching. If, however, the amount of sodium hydroxide used in preparation is kept very low, in fact is insufficient by itself to give complete treatment, then a small quantity of Calgon solution will suppress any haze or cloudiness that would have developed. Tidal waters vary considerably in their sodium hydroxide requirements and further work is necessary to see if they can be adapted to the simplicity of the technique as it applies to sewages and effluents.

SUMMARY—

A simple, rapid, direct nesslerisation method of estimation of ammonia nitrogen in sewages and effluents gives results comparable with those obtained by controlled distillation.

Sodium hydroxide, normally used in the preparation of samples, can give rise to serious errors: its use, together with the filtration stage, is avoided by using a small quantity of sodium hexametaphosphate (Calgon).

The method has limitations with tidal river waters.

Thanks are due to Dr. R. J. Stephenson, Mr. P. E. L. Farina and Mr. H. G. Haynes for their part in the experimental work, and to Mr. C. J. Regan, Chemist-in-Chief of the London County Council, for advice and encouragement.

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LONDON COUNTY COUNCIL
PUBLIC HEALTH DEPARTMENT
SOUTHERN OUTFALL WORKS
CROSSNESS, LONDON, S.E.2

J. E. HOULIHAN
March, 1949

CONTAMINATION OF WATER BY TRICHLOROETHYLENE

CASES of contamination of wells by trichloroethylene have come to our notice. In the first, the well was situated beside a factory that used large quantities of trichloroethylene as a solvent. During a fire at the factory a tank of the liquid burst and the ground was saturated with the solvent. After more than four years the water in the well still had an odour of trichloroethylene and the well had to be abandoned. The well was sunk in gravel only about 20 feet from a river and one might have expected that the movement of water through the gravel would have removed the contaminant.

In the other case, the well was situated 150 to 200 yards from a pit in an open field where waste trichloroethylene had been dumped. It was in valley gravel and in the direct line of flow towards the river. The water in it had a slight odour of trichloroethylene and was said to cause stomach disorders, giddiness, etc. The amount of trichloroethylene in the water was found to be 18 parts per million when estimated by the following method, a modification of the Fujiwara pyridine - sodium hydroxide reaction.

From these two cases it is evident that contamination by compounds of this nature is likely to be very persistent and there is some evidence of toxicity at very low concentrations.

METHOD—

Standard solution—A convenient standard can be prepared by first dissolving 1 ml. of commercial trichloroethylene in alcohol and making up with alcohol to 100 ml., and then diluting 1 ml. of this solution to 500 ml. with water, to give a solution containing 20 parts per million.

Procedure—Place 5 ml. of the sample in a test tube, add 2 ml. of colourless pyridine and 5 ml. of a 50 per cent. w/v solution of sodium hydroxide in water, shake thoroughly and stopper with cotton wool. Place the tube, and a similar tube containing the standard solution treated in the same way, in a boiling water-bath for 5 minutes. Cool and compare the orange colour obtained in the supernatant liquid with that of the standard. For a more accurate estimation prepare a series of tubes containing different amounts of trichloroethylene and match with the sample treated in parallel. It should be possible to estimate 5 parts per million with ease and the method would probably detect 1 part per million.

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Jacobs, M. B., *Analytical Chemistry of Industrial Poisons, Hazards and Solvents*, Interscience Publishing Co., New York, 1941.

F. A. LYNE
T. McLACHLAN
March, 1949

ABBEY GATEWAY
READING

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Direct Determination of the Volume Occupied by the Husk in the Analysis of Malts. J. R. Gwilt (*J. Inst. Brew.*, 1947, **53**, 152–154)—In the standard method for the determination of extract in brewing malts in this country (*Ibid.*, 1933, 517) a volume of 15 ml. is allowed for husk and other insoluble matter present in 50 g. of malt.

Warren (*Brew. Trade Rev.*, 1924, **38**, 148) has shown the allowance of 15 ml. to be too high. Bishop (*J. Inst. Brew.*, 1934, 187) allows for variation in the volume occupied by the insoluble solids and finds an average of about 10 ml. for British two-rowed malts, and later (*Ibid.*, 1944, 140) gives a method for correcting apparent extract to find the true extract. In the present work the volume occupied by the grains is measured directly.

Method—Mash 50 g. of malt as in the Institute of Brewing's standard method (*Ibid.*, 1933, 517), and at the end of the mashing period transfer the material quantitatively to a litre-beaker and heat it to boiling-point. Allow the grains to settle and decant the supernatant liquid through a 24-cm. Whatman No. 12 fluted paper. Repeatedly extract the grains with boiling water and wash by decantation until all soluble matter has been removed. (In the investigation this was checked by determination of the sp.gr. of the washings.) Rinse the residue on the paper back into the beaker with hot water, remove most of the water by boiling, and dry the residue, first to apparent dryness at 98°

to 100° C., then by two evaporations with acetone and finally at 100° C. to approximately constant weight. Weigh the dried grains and transfer them quantitatively to a 150-ml. graduated flask containing about 80 ml. of acetone, swirl the flask to remove air bubbles, dilute to the mark with acetone, and weigh. Weigh the flask empty and full of acetone and again full of water (to obtain the density of the acetone), making all determinations at 68° F. as a convenient standard temperature. Thus, the weight of acetone displaced by the grains can be calculated and hence its volume, *i.e.*, the volume occupied by the grains.

Heron (*Ibid.*, 1902, 668) gave 13 and 17 ml. as the outside limits for the volume of insoluble solids in the malts he had examined, whereas Brown (*Ibid.*, 1943, 195; *Analyst*, 1943, **68**, 309) found the volume to be 9·5 to 10·7 ml. for British two-rowed malts. Determinations by the method given above with some British two-rowed malts gave results ranging from 9·7 to 10·3 ml., *i.e.*, in good agreement with Brown's figures. Hence, although the standard method of analysis gives results consistent among themselves, it is confirmed that the apparent extract for average brewery malts is nearly 1 lb. low compared with other extract-yielding materials. A correction for a wide range of malts can be obtained from Bishop's tables (*loc. cit.*).

For comparison, determinations were made with crystal (12·6), brown (12·0) and chocolate (13·4) malts and with oat malt (19·0) and with unmalted flaked barley (12·1), the results in brackets being the volume (ml.) of the grains from 50 g. of the material. A. O. JONES

Data for the Oils of Some Common Oleaginous Seeds. G. Loew (*Industria y Quim.*, 1948, **10**, 5–6)—Healthy onion seeds (*Allium Cepa*) contained 8 per cent. of moisture and 17·90 per cent. of fatty material. Oil obtained from the crushed seeds by expression, and by extraction with naphtha gave, respectively, the following data: d^{15} 0·9354, 0·9289; n^{25} 1·4738, 1·4730; solidifying-point −15° C., −15° C.; $\alpha^{2}_{\text{D (ethyl sulphate)}}$ −1·8, −1·5; deviation (Jean) +30°, +26·8°; acidity as oleic acid 1·78, 1·92 per cent.; saponification value 187·7, 197·5; iodine value (Rosenmund and Kuhnhenn) 107·7, 111·9; unsaponifiable matter (Spitz and Hönig) 2·37, 1·20 per cent.; modified Bellier test—became turbid on cooling from 60° C. to 11°, 24° C., respectively. A. F. STURGESS

South African Fish Products. Part XXIX. The Composition of the Liver Oil of the Soupfin Shark (*Galeorhinus canis*, Rond.). M. L. Karnovsky, A. W. Lategan, W. S. Rapson, and H. M. Schwartz (*J. Soc. Chem. Ind.*, 1948, **67**, 193–196)—The iodine value of the liver oil of the mature fish increases with the oil content of the liver. This is due to the greater unsaturation of C_{16}, C_{18}, C_{20}, and C_{22} acids.

In the mature fish vitamin A, α-glyceryl ethers, and sterols are major components of the unsaponifiable matter; sterols predominate in the foetus. The unsaponifiable matter of four samples of liver oil from mature fish was from 1·99 to 7·84 per cent. Results are given in the accompanying table.

	I	II	III	IV	V
Liver oil from	Mixed oils	Fat females	Thin females	Male	Foetus
Oil in liver, %	—	83·0–83·2	26·2–36·0	60·2	46·1
Unsap. in oil, %	1·99	2·52	7·12	7·84	8·68
$E^{1\%}_{1\,\text{cm.}}$ 328 mμ. of oil	3·78	9·16	48·4	63·6	0·15
Neovitamin A as % of total vitamin A	30·9	34·1	24·1	29·0	—
Composition of unsaponifiables.					
Total vitamin A, %	11·2	21·5	39·7	47·7	0·1
Cholesterol, %	28·7	19·6	24·9	6·5	88·3
α-Glyceryl ethers,* %	21·7	20·5	15·7	17·8	3·4
Squalene, %	3·8	1·4	1·1	0·6	0·5
Satd. hydrocarbons, %	2·2	—	0·7	—	0·2
Total	67·6	63·0	82·1	72·6	92·5
Sapon. value of unsap. after acetylation**	195·5	221·3	—	199·0	162·8
do. (calc.)**	134·9	137·9	165·6	161·4	139·7
Fatty alcohols in unsap., % (calc. as oleyl alcohol)	29·0	39·9	—	17·9	11·1

* Calculated as selachyl alcohol.

** Calculated as mg. of KOH required to saponify acetates from 1 g. of unsaponifiable.

E. B. DAW

Biochemical

Oxidase Activity in Potato Tubers. IV. Quantitative Method for the Determination of Peroxidase. J. S. Wallerstein, R. T. Alba, M. G. Hale, and H. Levy (*Biochim. et Biophys. Acta*, 1947, **1**, 327–334)—A method is described for the determination of peroxidase by a reaction with *o*-phenylenediamine in presence of hydrogen peroxide under standard conditions. The reaction is stopped by adding acetone, the mixture is extracted with butanol, and the butanol extract assayed colorimetrically and fluorometrically.

Method—Wash and peel the potatoes, cut into dice of about 1-ml. volume and wash briefly in running water. Homogenise a suitable weight of dice (normally 40 g.) with water in a Waring blendor at high speed in a total volume of 300 ml. After 60 sec. reduce the speed of the blendor, and add a few drops of caprylic alcohol. Pipette 20 ml. of the homogenate from the blendor for the test. Where indicated, blanch the dice in a colander by placing in boiling water for the required time, and immediately cool in ice-water. Prepare purified horse-radish peroxidase by the method of Willstaetter and Stoll (*Annalen*, 1918, **416**, 21).

Procedure—Add 100 ml. of *o*-phenylenediamine solution (made by dissolving 25 g. of *o*-phenylenediamine dihydrochloride in water, diluting to 2 litres and adjusting the pH to 5·0 with sodium hydroxide) to 200 ml. of water, and stir slowly in a beaker. Add 0·7 ml. of 30 per cent. hydrogen peroxide and 20 ml. of homogenate. Stir continuously for 4·5 min., remove 50 ml. of the reaction mixture and, at 5 min., add 50 ml. of acetone to stop the reaction. Pipette 5 ml. of the acetone mixture into 95 ml. of water and 100 ml. of butanol in a separating funnel. Shake vigorously, separate the butanol layer, filter through a Whatman No. 5 filter paper, and measure in a Klett - Summerson colorimeter, using standard Klett test tubes, with a number 42 blue filter against a butanol blank. Also measure the fluorescence on 2 ml. of the butanol filtrate after mixing thoroughly with 48 ml. of butanol, against a standard of 1 part of fluorescein in 4 million parts of 0·01 *N* sodium hydroxide, using a Pfaltz and Bauer fluorophotometer. For comparative purposes a purpurogallol peroxidase assay was carried out on aliquots of the same batch of homogenate used for the *o*-phenylenediamine test. The technique was a modification of the method of Willstaetter and Stoll as applied by Stern and Kegeles (unpublished communication, Yale University, 1941). The method depends on the extraction by ethyl acetate of purpurogallol formed from pyrogallol, and photometric determination of the optical density of the extract with correction for the tyrosinase blank.

Results—Results by the *o*-phenylenediamine method correspond closely to the Willstaetter pyrogallol procedure. Reproducibility is comparable in the two methods, whereas sensitivity and ease of assay is substantially greater with the *o*-phenylenediamine procedure. Under the conditions of assay the amount of colour and fluorescence developed is linearly proportional to time for about 15 min., and thereafter shows a sharp decline. The temperature optimum for the reaction is about 60° C. J. S. HARRISON

Infra-Red Analysis of Crystalline Penicillins. R. B. Barnes, R. C. Gore, E. F. Williams, S. G. Linsley, and E. M. Petersen (*Anal. Chem.*, 1947, **19**, 620–627)—A method, based on the presence in the infra-red spectrum of characteristic absorption bands, is presented for the analysis of each of the different varieties of penicillin. Owing to the insolubility of the salts of penicillin in all the solvents that are of use in infra-red measurements, and to the risk of loss and degradation in converting the salt to the free acid and transferring to a suitable dry solvent, it was necessary to record the spectrum of the crystalline salts.

The sample was prepared by mulling a small quantity (0·5 to 3·0 mg.) of crystalline sodium penicillin with liquid petrolatum (Nujol) directly upon the rock-salt plates of the absorption cell. A recording spectrometer (Perkin - Elmer Model 12-B) was used. Many of the absorption bands found are associated with the crystal structure of the material and the nature of the cation of the salt affects the spectrum; the results given here are for crystalline sodium salts only. All types of penicillin show a characteristic absorption band at 1770 cm.$^{-1}$. The frequencies of bands characteristic of individual types and sufficiently free from mutual overlapping to be used for analysis are: G (benzyl) 703 cm.$^{-1}$; X (*p*-hydroxybenzyl) 831 and 1220 cm.$^{-1}$; F (Δ^2-pentenyl) 971 cm.$^{-1}$; K (*n*-heptyl) 1330 cm.$^{-1}$; dihydro F (*n*-amyl) 715 and 1166 cm.$^{-1}$

For quantitative analysis it is necessary to know the thickness of the sample under examination. In effect, this is done by mixing intimately with the penicillin a solid internal standard in known concentration. The standard should have a strong absorption band convenient to the spectral regions of interest, but have no absorption interfering with the analytical bands; it should be easily weighable and mullable with the sample, and easily obtainable in a pure state. As internal standard, DL-alanine was chosen, and measurements were made on the band at 851 cm.$^{-1}$

Procedure for penicillin G—Establish a calibration curve as follows. Weigh out 15 mg. of thoroughly ground, pure penicillin G and 7·5 mg. of DL-alanine. Mix the two dry with a small mortar and pestle. Place a drop of Nujol on one of the polished rock-salt plates of the absorption cell, and add about 10 mg. of the dry mixture. Using the second rock-salt plate of the absorption cell, mull the sample thoroughly. Adjust the cell thickness to give about 50 per cent. transmission at 851 cm.$^{-1}$ Set the spectrometer at 925 cm.$^{-1}$ with the cell in the beam, and adjust the slits and amplification factor to give a full-scale deflection. Record the spectrum from 925 to 775 cm.$^{-1}$, the zero being determined at the beginning and end of the run. Set the spectrometer at 760 cm.$^{-1}$, adjust for full-scale deflection and record from 760 to 695 cm.$^{-1}$ Note the slit settings and amplification factors, which must be duplicated in all successive runs. From the record of the spectrum, measure I and I_0 (the intensities, respectively, of the transmitted and incident radiations) at 703 cm.$^{-1}$ and 851 cm.$^{-1}$ Obtain I_0 by linear interpolation of the background radiation. Repeat on samples successively diluted with an inert solid, such as magnesium oxide, and plot a calibration curve of $R = \log (I_0/I)$ at 703 cm.$^{-1}$ $\div \log (I_0/I)$ at 851 cm.$^{-1}$ *versus* percentage of penicillin G. Using this calibration curve, analyse the unknown by the same procedure. An accuracy to within ±2 per cent. is claimed.

Other types of penicillin are analysed similarly by using the appropriate absorption band. In some determinations, the values of I_0 were determined by using the cell in - cell out technique.

Interference in the analysis may be caused by impurities in the penicillin if these have absorption bands close to those used for analysis. The band at 703 cm.$^{-1}$ in sodium penicillin G is due to the monosubstituted phenyl group, and any impurities, such as sodium phenyl acetate, having this grouping will interfere with the analysis of penicillin G. The presence of such impurities may be detected by the presence of additional bands in the spectrum, and for this reason the complete spectrum of a sample is recorded before analysis.

Effects due to crystal orientation were found with one sample of penicillin G, the crystals of which were in the form of flat plates. On pressing the Nujol mull between the rock-salt plates, orientation of the crystals took place; analysis gave a low value. Orientation was revealed by rotating the absorption cell at an angle to the beam of radiation. Orientation affects only some of the absorption bands and its presence can be detected by an abnormally low value of the ratio log (I_0/I) at 805 cm.$^{-1}$ ÷ log (I_0/I) at 900 cm.$^{-1}$ Orientation can be prevented by more thorough grinding of the sample, and a grinding time of 5 min. is recommended. G. H. TWIGG

Polarographic Estimation of Steroid Hormones 4. Determination of 3 (α)- and 3 (β)-hydroxy-17-ketosteroids. W. R. Butt, A. A. Henly, and C. J. O. R. Morris (*Biochem. J.*, 1948, **42**, 447–452)—A method is given for the separation of 3 (α)- and 3 (β)-hydroxy-17-ketosteroids in urine. The urine is first hydrolysed and extracted (Barnett, *et al.*, *ibid.*, 1946, **40**, 445).

Reagents—(1) *Digitonin solution*—Use a 1 per cent. solution of B.D.H. digitonin in warm 90 per cent. ethanol. The digitonin can be kept in solution by storing the reagent at 37° C. (2) *Pyridine*—Dry over barium oxide and distil frequently.

Procedure—Put into a graduated centrifuge tube a benzene solution of urine extract containing 1 to 1·5 mg. of 17-ketosteroid. Evaporate to dryness under reduced pressure. Add 0·75 ml. of digitonin solution and quickly heat to boiling over a microflame to dissolve as much material as possible. Stopper the tube and keep overnight in a refrigerator. Add in portions, and with stirring, 10 ml. of peroxide-free ether. Allow the precipitate to flocculate before adding the last 2 to 3 ml. Centrifuge for 5 min. at 2000 to 3000 r.p.m. and decant the supernatant liquid into a 50-ml. separating funnel. Wash the precipitate three times with 5-ml. portions of ether, stirring and centrifuging as before. Wash the bulked liquid three times with 5-ml. portions of water, transfer to a small flask, evaporate the ether under reduced pressure, and dry the residue *in vacuo* over calcium chloride for 1 hr. This constitutes the α-fraction.

Dissolve the residue in the centrifuge tube in 0·25 ml. of dry pyridine and place in a water-bath at 60° to 70° C. for 3 min. Cool, and then add 5 ml. of ether in small quantities as before. Centrifuge for 5 min. and transfer the supernatant liquid to a small separating funnel. Wash the residue in the centrifuge tube with pyridine and ether and finally wash twice with 5-ml. portions of ether. Wash the combined extracts twice with 5-ml. portions of 2 *N* sulphuric acid, and then three times with 5-ml. portions of water. Transfer to a small flask and dry the residue exactly as above. This constitutes the β-fraction.

With normal urines use about one-tenth of the α-fraction and the whole of the β-fraction. Oxidise with permanganate - periodate, treat with Girard's reagent T, and take a polarogram (*Idem, ibid.*). W. S. WISE

Agricultural

Determining Small Amounts of Calcium in Plant Materials. A Colorimetric Method. E. H. Tyner (*Anal. Chem.*, 1948, **20**, 76–80)—*Reagent*—*Chloroanilic acid solution*—Add 1 g. of chloroanilic acid to 500 ml. of distilled water, dissolve by warming to 50° C., cool, and dilute to 1 litre.

Procedure—Place 1 g. of the finely ground plant material in a 50-ml. Pyrex beaker and heat to 450° C. in a muffle furnace. Moisten the cold ash with a few drops of distilled water, dissolve in 5 ml. of diluted hydrochloric acid (1 + 3), and evaporate to dryness. Add 5 ml. of 0·1 *N* acetic acid, warm for a few minutes, cool, transfer to a 100-ml. graduated flask, and dilute to the mark. Filter the liquid into a dry Erlenmeyer flask. Pipette a portion of the filtrate containing from 0·4 to 1·1 mg. of calcium into a 50-ml. graduated flask, dilute to 20 ml. and add from a pipette 10 ml. of a 0·1 per cent. chloroanilic acid solution. Allow to stand for at least 3 hr. Dilute to 50 ml., mix, and filter the liquid into a dry flask. Using a spectrophotometer and light of wavelength 550 mμ., compare the light transmission of the liquid with that of a water blank. To calibrate the instrument treat solutions of known calcium content with 10 ml. of the chloroanilic acid solution and proceed as from that point in the determination.

Iron, aluminium, barium, strontium, and manganese in the amounts usually present in plants do not interfere. As magnesium chloroanilate is occluded in the calcium chloroanilate precipitate the presence of magnesium may give high results. Results obtained by the method are usually higher than those obtained by the standard volumetric method (*Assoc. Off. Agric. Chem.*, *Official and Tentative Methods of Analysis*, 5th Ed., 1940, p. 127). B. ATKINSON

New Method for Determination of Small Amounts of Molybdenum in Plants. C. S. Piper and H. S. Beckwith (*J. Soc. Chem. Ind.*, 1948, **67**, 374–379)—The method developed for microgram amounts of molybdenum involves preliminary extraction of molybdenum with cupferron and chloroform and the subsequent formation of the complex of molybdenum with dithiol (4-methyl-1 : 2-dimercaptobenzene). The optimum pH for extraction of molybdenum from acid solutions containing citrate is 0 to 0·5, and precipitation of the molybdenum - dithiol complex in 15 to 25 per cent. (v/v) sulphuric acid prevents interference by tungsten. For the extraction of the dithiol complex, amyl acetate proves to be a better solvent than amyl alcohol.

METHOD—*Reagents*—*Dithizone solution*—Dissolve 0·25 g. in 600 ml. of carbon tetrachloride, warming to 50° C. to assist solution, extract the dithizone as the ammonium salt by shaking with 350 ml. of water containing 3 to 4 ml. of concentrated aqueous ammonia and wash the separated aqueous layer with three 75-ml. portions of re-distilled carbon tetrachloride, discarding the carbon tetrachloride layer each time. Add 600 ml. of re-distilled carbon tetrachloride, slightly acidify the aqueous layer with re-distilled hydrochloric acid, shake thoroughly, and wash the carbon tetrachloride solution of dithizone in another separator with three 150-ml. portions of water, discarding the aqueous layer each time. To the carbon tetrachloride solution of dithizone in a stoppered Pyrex reagent bottle add 750 ml. of carbon tetrachloride and store at 2° to 5° C.

Cupferron solution—Prepare fresh daily by dissolving 3 g. in 50 ml. of water, and filter if necessary.

Dithiol solution—Dissolve 1 g. of dithiol from a freshly opened tube in 500 ml. of a 1 per cent. solution of sodium hydroxide. The dithiol dissolves more readily if the tube is warmed to 30° to 35° C. before opening. When completely dissolved add thioglycollic acid from a pipette with constant stirring until a slight haze forms (8 to 9 ml.). Completely fill several 50- or 100-ml. stoppered reagent bottles (preferably of Pyrex glass) with the reagent and store at 2° to 4° C. The reagent is then stable for about 3 months.

Sodium perchlorate solution—Dissolve 550 g. of pure sodium perchlorate in water and dilute to 1 litre. Make the solution alkaline to phenol red (used externally) with sodium hydroxide solution, filter if necessary, and shake with 20- to 30-ml. portions of dithizone solution (*supra*) until no more heavy metals appear to be extracted. Shake finally with 50 ml. of carbon tetrachloride, separating as completely as possible and discarding the carbon tetrachloride layer. Acidify with a few drops of sulphuric acid and store in a Pyrex bottle. The excess of dithizone remaining in the solution does not interfere with its use.

Ammonium citrate solution, 12 per cent.—Dissolve 100 g. of citric acid in water, neutralise with aqueous ammonia solution to pH 7 to 8, and dilute to 1 litre. If this reagent is used for copper determinations, purify it with dithizone as described for sodium perchlorate.

Procedure—To 2 to 4 g. of the material in a Kjeldahl flask add 4 ml. of perchloric acid and enough nitric acid to ensure oxidation of all the organic matter. The perchloric acid may be replaced by 8 ml. of sodium perchlorate solution (*supra*) and an additional ml. of sulphuric acid. Add 2 to 5 ml. of sulphuric acid (preferably 5 ml. if it does not interfere with subsequent work) and maintain the presence of at least 2 ml. of sulphuric acid to prevent local overheating with consequent risk of explosion after the nitric acid has been expelled. Mix well, and heat the flask at low heat (120 to 150 watts) on a hot-plate or over a Bunsen burner turned very low. When dense brown fumes appear, remove the flask from the heater for 5 min., then resume digestion slowly and at low heat until dense fumes of sulphuric acid appear. Continue digestion for 5 to 10 min. and finally for 1 to 2 min. at the full heat of the plate (500 to 600 watts). If the liquid is not now colourless, add 1 or 2 ml. of nitric acid and heat to fuming. Dilute the digest with water, add ammonium citrate solution, neutralise to pH 3, and extract with dithizone solution to remove copper. One extraction suffices unless the copper content is being determined. To the aqueous residue add 8 ml. of diluted sulphuric acid (1 + 1), thereby reducing the pH to 0·3 to 0·5, and shake well. Add 1 to 1·5 ml. of 6 per cent. cupferron solution and shake again to precipitate all iron and molybdenum, using the larger amount if the iron content appears to be high. Add 10 ml. of chloroform and shake for 40 sec. to extract the metal-cupferron complex. After 3 to 5 min. separate the chloroform phase, add 0·5 ml. more of the cupferron solution and 10 ml. of chloroform, shake for 30 sec., and separate after allowing to stand as before. If this second extract does not show a blue tinge, repeat the extraction with 3 drops of cupferron solution and 5 ml. of chloroform. Remove the solvent from the combined extracts by boiling in a micro-Kjeldahl flask containing 2 glass beads. Remove the flask from the heater, add 3 ml. of concentrated sulphuric acid, and digest for 1 to 2 min. until all the chloroform vapour has been expelled. Add 5 drops of perchloric acid and again digest until the liquid is nearly clear. Remove the flask from the heater to allow the condensate to rinse down volatilised matter in the neck, and, if necessary, rinse the neck of the cooled flask with 1 ml. of water. Re-heat until the neck is perfectly clean (5 min.).

Dilute the cold digest with 5 ml. of water, boil gently for 1 to 2 min. to dissolve all the ferric sulphate, transfer the cold solution to a 25-ml. graduated test mixer with three 2- or 3-ml. portions of water, adjust the volume to 17 ml., mix thoroughly and, when the mixture is quite cold, add 15 drops of the dithiol reagent, shake immediately twice and allow to stand for 30 min. to ensure complete precipitation of molybdenum. Add, by means of a pipette, 7·5 ml. of *iso*amyl acetate (b.p. 135° to 142° C.), shake thoroughly to extract the complex, and set aside for 4 to 6 hr. The colour is stable for at least 24 hr. Remove the amyl acetate phase and determine the intensity of the colour in a photoelectric colorimeter, using a waveband of 670 to 690 mμ. With a filter photometer use a filter combination of Ilford spectral orange (607) and spectral red (608).

To prepare the calibration curve add to suitable amounts of a standard molybdenum solution in a series of micro-Kjeldahl flasks 0·5 mg. of iron in the form of ferrous sulphate solution, 3 ml. of concentrated sulphuric acid and 2 glass beads and digest until fuming occurs. Cool, dilute, transfer to 25-ml. test mixers and develop the colour as already described. Make a blank determination, adding 0·5 mg. of iron during the digestion with nitric, sulphuric, and perchloric acids.

The transmission curve for the molybdenum-dithiol complex shows strong absorption at 670 to 690 mμ. and at 430 to 440 mμ., the former wavelength range being the more suitable for photometric measurements in the presence of other metals.

In the latter range, tin, when present, causes interference. Satisfactory determinations can be made in the range of 0·2 to 25 µg. of molybdenum. For larger amounts it is desirable to use 0·5-cm. cells or to increase the volume of amyl acetate used to extract the dithiol complex.

Of the other elements extractable by cupferron and chloroform at pH 0·5, copper, tin, and tungsten are the only ones reported to give colour complexes with dithiol. The copper complex collects as a bluish-black precipitate at the interface, and 19 µg. of copper give an absorption comparable with 2 µg. of molybdenum. It is desirable therefore to remove most of the copper by a single extraction with dithizone solution as described. Tin gives a bright pink precipitate with dithiol and is readily detected in more than trace amounts. It rarely occurs naturally in plant material in amounts sufficient to interfere, although material stored in contact with tin plate may contain 50 p.p.m. or more. When dissolved in amyl acetate the tin complex gives a very pale yellow solution, its effect on the transmission of the molybdenum - dithiol complex being negligible at 670 to 690 mµ. When tin is present in undue amounts (> 100 µg.) it can be removed, together with iron, by a preliminary extraction with cupferron and chloroform at pH 4·5. Tungsten gives a bluish-green precipitate with dithiol but not in the strongly acid conditions of this method. Iron in 2-mg. amounts (*i.e.*, more than is likely to be present from plant material) causes no significant error. Titanium, vanadium, zirconium, thorium, and uranium are extractable by cupferron, but give no coloured complexes with dithiol and have no effect upon the transmission of the molybdenum complex. A. O. Jones

Determining Total Replaceable Bases in Soils. C. J. Schollenberger (*Anal. Chem.*, 1948, **20**, 1121)—The residue, obtained by igniting the evaporated ammonium acetate "leachate" of a soil, according to Bray and Willhite (*Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 144), is sometimes grey to black and not completely soluble in boiling *N* hydrochloric acid. The insoluble residue can be dissolved, and its basicity included in the determination, by adding 2 to 3 drops of 30 per cent. hydrogen peroxide solution and allowing to stand for a few minutes before boiling. No error is incurred if the indicator, methyl red, for the back-titration of the excess of acid is in alcoholic solution. M. E. Dalziel

Water Analysis

Two New Total Alkalinity Indicators. M. Taras (*J. Amer. Water Works Assoc.*, 1948, **40**, 468–472)—Two indicators are recommended, particularly for alkalinities less than 150 p.p.m., *viz.*, *A*, disodium 4 : 4′-*bis*(*m*-tolyltriazeno)-2 : 2′-stilbenedisulphonate and *B*, disodium 4 : 4′-*bis*(*p*-methylaminophenylazo)-2 : 2′-stilbenedisulphonate. They give yellow to pale brown and orange to brown colour changes, respectively, at pH 5·4 to 5·2. Both change to mauve at pH 4·8, become purplish at pH 4·6 and a deeper purple up to pH 4·0. *B* is insoluble at the neutral point and gives rise to purple specks as the end-point is approached. *A* is prepared by tetra-azotisation of 4 : 4′-diaminostilbene-2 : 2′-disulphonic acid and coupling with *m*-toluidine; *B* is prepared by coupling the same azo compound with monomethylaniline (see *Anal. Chem.*, 1947, **19**, 339). The indicators are used in 0·1 per cent. solution. *A* is best dissolved in acetone. C. F. Herbert

Organic

Detection of Diphenylamine in Commercial Petrol. G. E. Mapstone (*Chem. and Ind.*, 1948, 807)—The possibility of detecting diphenylamine in petrol was examined by extracting the sample with a concentrated acid and then adding a strong oxidising agent. Concentrated hydrochloric and sulphuric acids and 70 per cent. phosphoric acid were tried, solid potassium dichromate and sodium nitrite being used as the oxidising agents. With sulphuric acid, side reactions interfered, even when the mixture was poured into water. With phosphoric acid the colours obtained ranged from yellow to red, whereas with hydrochloric acid they were green to blue. In general, sodium nitrite gave better results than potassium dichromate.

A study of the conditions of the test with concentrated hydrochloric acid and sodium nitrite led to the following procedure. Shake 10 ml. of the sample with 1 ml. of concentrated hydrochloric acid, add a small crystal of sodium nitrite, and shake. The presence of diphenylamine is indicated by a blue colour in the acid layer. The test is not as sensitive as that with ammonium vanadate (the basis of the official test) and requires the presence of 40 to 50 p.p.m. of diphenylamine to yield a light blue colour. It has the advantage of dispensing with the preliminary washing of the petrol with sodium hydroxide solution, and of using reagents immediately available without preparation.

The test was compared with the original form of the official test (*Ibid.*, 1948, 383). This form of the official test, however, has now been replaced by an improved modification. A. O. Jones

Determination of *cyclo*Pentadiene and Methyl*cyclo*pentadiene in Admixtures with other Hydrocarbons. J. S. Powell, K. C. Edson, and E. L. Fisher (*Anal. Chem.*, 1948, **20**, 213–215)—An apparatus for the quantitative depolymerisation of the dimers of *cyclo*pentadiene and methyl *cyclo*pentadiene is described, together with a method for determining the monomers that is based on measurement of the optical densities of solutions of the fulvenes formed from the monomers by reaction with acetone and benzaldehyde. The determination is unaffected by other unsaturated or aromatic hydrocarbons.

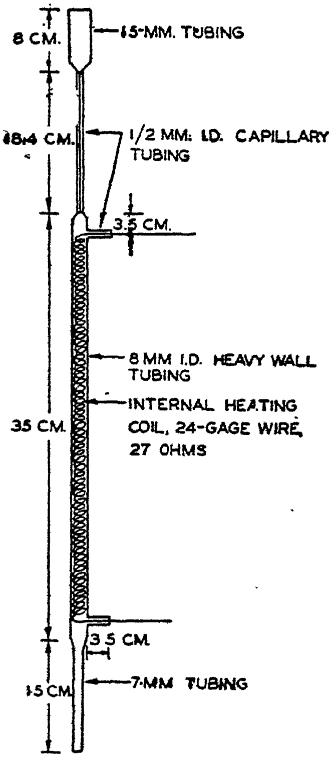
Method—The depolymerisation apparatus is shown in Fig. 1. By means of a preliminary trial adjust the power input of this apparatus so that the depolymerised sample vapour leaves the heating coil at 340° to 360° C.

Preparation of sample—Data for suitable flasks, dilutions, and volumes of sample are given in Table I. Dry the sample with sodium sulphate. Plac approximately 20 ml. of toluene (*C.P.*) in an appropriate volumetric flask and weigh. Pipette a suitable volume of the sample into the flask, reweigh, and dilute to the mark with toluene.

TABLE I

Approximate percentage w/w of dimer in sample	Required dilution of sample	Volume of mixture to depolymerise ml.	Volume of receiver ml.
0·0 to 0·9	None	15	50
0·8 to 2·8	None	5	50
2·6 to 9·0	3/10	5	50
9·0 to 28	1/5	5	100
28 to 90	3/50	5	100

Place the outlet tube of the depolymerisation apparatus in a second volumetric flask acting as receiver and containing 20 ml. of toluene and cooled by acetone and solid carbon dioxide. Pass the diluted sample through the apparatus and wash through with 5 ml. of toluene. Remove the receiver, allow its contents to come to room temperature, and then dilute to the mark with toluene. Aliquot parts of the solution are used in the determination.


8 CM.
15-MM. TUBING
18.4 CM.
1/2 MM. I.D. CAPILLARY TUBING
3.5 CM.
8 MM I.D. HEAVY WALL TUBING
INTERNAL HEATING COIL, 24-GAGE WIRE, 27 OHMS
35 CM.
3.5 CM
7-MM TUBING
15 CM

Determination of cyclo*pentadiene and methyl*cyclo*pentadiene monomers*—The depolymerised sample as prepared has the correct dilution for reaction with acetone, but requires diluting 1 to 5 for the reaction with benzaldehyde.

Prepare four 125-ml. glass-stoppered bottles as follows.

In (1) and (2), mix 5 ml. of acetone and 5 ml. of alcoholic potassium hydroxide solution (5 g. in 100 ml. of 95 per cent. ethanol). In (3) and (4), mix 5 ml. of a 20 per cent. v/v solution of C.P. benzaldehyde in 95 per cent. alcohol and 5 ml. of alcoholic potassium hydroxide solution. Prepare bottles (2) and (4) to serve as blanks by adding 10 ml. of 4 per cent. acetic acid solution to each. Immerse all four bottles in a water-bath and allow them to reach the bath temperature of 32° C. Add 5 ml. of the correctly diluted sample to each bottle and swirl the liquids without allowing them to touch the stoppers. Add 10 ml. of 4 per cent. acetic acid to bottle (1) after exactly 15 min., and to bottle (3) after exactly 20 min. Add 20 ml. of toluene to each bottle, mix thoroughly, decant the hydrocarbon layers into 50-ml. beakers, add approximately 1 g. of sodium sulphate to each beaker and cover with watch glasses. After dehydration, determine the optical densities of the toluene solutions with a spectrophotometer at a wavelength of 425 mμ. and with a slit width of 0·03 mm.

Calculation of results—First calculate the extinction coefficients, K, from the observed optical densities, D, after allowing for the densities of the blanks, from the equation $K = D/C\,L$, where C is the concentration of the sample in grams per litre, and L is the optical path in cm. The weight per cent. of *cyclo*pentadiene then equals $100\,(k_1K_1 - k_2K_2)$, and that of methyl*cyclo*pentadiene $100\,(k_3K_2 - k_4K_1)$, where K_1 and K_2 are the extinction coefficients for the acetone and benzaldehyde reaction products, respectively. The constants k_1 to k_4 are determined by replication of the analysis with pure *cyclo*pentadiene and methyl*cyclo*pentadiene separately, and determining the extinction coefficients for the two compounds in both reactions. Then, $k_1 = K_dA$; $k_2 = K_bA$; $k_3 = K_aA$; and $k_4 = K_cA$; and where $A = 1/(K_aK_d - K_bK_c)$, K_a refers to *cyclo*pentadiene and K_b to methyl*cyclo*pentadiene in the acetone reaction, and K_c to *cyclo*pentadiene and K_d to methyl*cyclo*pentadiene in the benzaldehyde reaction, respectively.

Results—The results of analyses on synthetic mixtures of the hydrocarbons show that the method is accurate; the greatest deviation from the correct value over a range of 8 to 21 per cent. of *cyclo*pentadiene and 0·4 to 10 per cent. of methyl*cyclo*pentadiene, was 0·3 per cent.

Indene, which also condenses with acetone and benzaldehyde to form fulvenes, reacts too slowly with acetone to cause appreciable interference, but, owing to its reaction with benzaldehyde, does introduce an error, in the methyl*cyclo*pentadiene content, of approximately one-fourth of the indene content. W. C. Wake

Spectroscopic Determination of cyclo-Pentadiene and Methylcyclopentadiene. J. S. Powell and K. C. Edson (*Anal. Chem.*, 1948, **20**, 510–511)—An ultra-violet absorption method is described for determining *cyclo*pentadiene and methyl*cyclo*pentadiene in presence of each other. The interference of aromatic hydrocarbons and conjugated di-olefines is allowed for by comparison of the absorption of monomeric and dimeric forms of the two substances. The absorption peak for *cyclo*pentadiene is at 240 mμ. and that for methyl*cyclo*pentadiene at 247 mμ. The absorption of the dimers of both substances is very small and is negligible at 240 mμ. and beyond. The absorptions determined are those of solutions in *iso*octane at 240 and 258 mμ.

Preparation of sample—Pipette 5 ml. of sample into a tared 100-ml. volumetric flask and weigh. Dilute to the mark with *iso*octane that has been purified by passage through a chromatographic column of silica gel until it shows no absorption of ultra-violet light at 240 mμ. Place the outlet tube of the depolymerisation apparatus (see previous abstract) in a 100-ml. volumetric flask containing 30 ml. of *iso*octane. Cool the flask with acetone and solid carbon dioxide. Transfer 5 ml. of the diluted sample to the funnel of the depolymerisation apparatus and, after depolymerisation, wash through with 5 ml. of *iso*octane. Remove the receiver, allow its contents to reach room temperature, and then dilute to the mark with *iso*octane.

If aromatic hydrocarbons or conjugated di-olefines are present, prepare a blank by heating the sample at 100° C. for 6 hr. in a closed container capable of withstanding a pressure of 10 atmospheres. This treatment dimerises the *cyclo*pentadiene and methyl*cyclo*pentadiene.

Dilute a portion of the sample and of the dimerised material, if a blank is necessary, according to Table I.

Table I

Weight of *cyclo*pentadienes in sample	Dilutions	
	For measurement at:—	
%	240 mμ.	258 mμ.
82–100	5/100	5/50
28–89	5/100	10/50
11–33	5/100	25/50
6–17	5/100	—
3–8	5/50	—
0–3	15/50	—

Determination of absorption—Determine the optical density of the diluted depolymerised sample at 240 and 258 mμ. with the spectrophotometer set at 100 per cent. transmittance on the diluted blank at each wavelength. If a dimerised blank is not necessary, then pure *iso*octane is used in its place.

Calculations—As in the method given in the previous abstract, determine the extinction coefficients (K); the weight per cent. of *cyclo*pentadiene is given by 100 ($k_1K_1 - k_2K_2$), and that of methyl*cyclo*pentadiene by 100 ($k_3K_2 - k_4K_1$), where K_1 and K_2 are the extinction coefficients at 240 and 258 mμ., respectively. As in the previous method, the values of the constants k_1 to k_4 are determined by experiment with pure compounds. The same equations apply, $k_1 = K_dA$; $k_2 = K_bA$; $k_3 = K_aA$; $k_4 = K_cA$; and $A = 1/K_aK_d - K_bK_c)$. In this case, however, K_a refers to *cyclo*pentadiene, and K_b to methyl*cyclo*pentadiene at 240 mμ., whilst K_c and K_d refer to the corresponding extinction coefficients at 258 mμ.

Results—The results of analyses on synthetic mixtures of the hydrocarbons show that the method is accurate, the greatest deviation from the actual value observed over a range of 32 to 91 per cent. of *cyclo*pentadiene and 9 to 68 per cent. of methyl*cyclo*pentadiene, was 1·3 per cent.

Indene also interferes in this second method and, if present to any extent, should be removed by fractional distillation. W. C. Wake

Polarographic Determination of Nitrobenzene. I. A. Korshunov, A. V. Ryabov, L. N. Sazanova, and A. S. Kirillova (*Zavod. Lab.*, 1948, **14**, 519–522; *cf.* I. A. Korshunov and A. S. Kirillova (*J. Gen. Chem. Russ.*, 1948, **18**, 785–792)—A polarographic wave suitable for the determination of nitrobenzene in concentrations not greater than 2 × 10^{-3} M in the supporting electrolyte is obtained in acid, neutral, or alkaline medium. The supporting electrolyte is a buffer—citric acid and disodium hydrogen phosphate, acetic acid and sodium acetate, hydrochloric acid and sodium chloride, or sodium hydroxide solution. The nitrobenzene is added to the buffer in the form of an alcohol, acetone, or dioxan solution, the volume of which does not exceed 5 per cent. of that of the buffer solution.

The half-wave potential at 20° ± 1° C. *versus* the saturated calomel electrode varies with the pH as follows:

pH	Half-wave potential (v.)
2	− 0·15
4	− 0·27
6	− 0·40
7	− 0·47
8	− 0·53
10	− 0·60
12	− 0·63
14	− 0·63

These figures were obtained with a capillary passing 2·75 mg. of mercury per sec., and having a drop-time of 3·2 sec. The nitrobenzene concentration was varied between 5 × 10^{-5} and 3 × 10^{-3} M without affecting the results. The relation between wave-height and concentration is not strictly linear. [The results do not differ greatly from the values of the "reduction potential" at 25° C. *versus* the normal calomel electrode, obtained by Shikata (*Trans. Faraday Soc.*, 1925, **21**, 42), but they differ appreciably from the half-wave potentials at 25° C. *versus* the saturated calomel electrode, obtained by Pearson (*Ibid.*, 1948, **44**, 683)].

A sharply-peaked maximum appears with concentrations of 10^{-3} M and above. Gelatin eliminates it and causes the half-wave potential to become more negative. Thus the half-wave potential at pH 1·0 is −0·1 v., but with a gelatin concentration of 0·01 per cent. it becomes −0·24 v., and with a concentration of 0·02 per cent., −0·27 v.

Because of the high volatility of nitrobenzene the indifferent electrolyte should preferably be de-oxygenated before the sample is added. The sample is therefore delivered from a special burette (see Heyrovsky, *Polarographie*, 1941, p. 259).

In presence of aniline or benzidine the determinations can be carried out with the hydrochlorides of these bases as indifferent electrolytes (4 volumes of aniline or 2 volumes of benzidine to 1 volume of concentrated hydrochloric acid). In nitric acid of 1·0 to 1·5 N or less, no added indifferent electrolyte is required.

The dinitrobenzene isomers in buffer solutions of pH 6·5 to 8·5 give two waves, the first coinciding with that of nitrobenzene, and the second at a potential 0·2 v. more negative. A mixture with nitrobenzene can be analysed by measuring the two wave-heights and adjusting the first for the amount of dinitrobenzene as found from the second.

G. S. SMITH

Advantages of Butyl Rubber in Organic Analysis. A. H. Corwin and C. Karr (*Anal. Chem.*, 1948, **20**, 1116)—The permeability of butyl rubber to air is only a tenth of that of natural rubber, and so butyl rubber is clearly superior for use in connections in the Dumas apparatus for the determination of nitrogen. Diffusion constants for natural, butyl, and silicone rubbers with respect to carbon dioxide and water vapour are given and, although not highly accurate, they indicate the advantage of connections of butyl rubber in carbon and hydrogen determinations. The constants are: for carbon dioxide, natural rubber $25{\cdot}2 \times 10^{-10}$, butyl rubber $0{\cdot}47 \times 10^{-10}$, and silicone rubber $25{\cdot}8 \times 10^{-10}$; and for water vapour, natural rubber $6{\cdot}41 \times 10^{-10}$, butyl rubber $4{\cdot}66 \times 10^{-10}$, and silicone rubber $36{\cdot}4 \times 10^{-10}$. M. E. DALZIEL

Inorganic

Iodimetric Micro-determination of Bromide and Iodide. E. Schulek (*Analyt. Chim. Acta*, 1948, **2**, 74–87)—Halogens react with alkali cyanides to form halogen ion and the corresponding halogen cyanides, from which free iodine is liberated easily and bromine more slowly, whilst chlorine cyanide is stable. By utilising this difference in behaviour, iodine and bromine can be separated from chlorine and determined iodimetrically. They can be determined in presence of one another within given limits.

Micro-determination of iodide—Chlorine method—Neutralise the solution (containing 1 to 10 µg. of iodide in 2 to 3 ml. in a small glass-stoppered vessel) to methyl red. The solution should be free from iron, manganese, and bromide. Add 2 to 5 drops of freshly prepared, saturated chlorine-water. After 5 min., add 2 drops of freshly prepared, 5 per cent. potassium cyanide solution and shake well three times, loosening the stopper, to ensure thorough mixing of the liquid. Acidify the mixture with 5 drops of 10 per cent. hydrochloric acid solution, and add 1 drop of 1 per cent. potato-starch solution and 3 drops of 20 per cent. potassium iodide solution. Cool the solution to 0° C. and after 10 min. titrate with 0·001 or 0·0005 N sodium thiosulphate from a micro-burette.

For 10 to 150 µg. of iodide, use a volume of 8 to 10 ml. in a 50-ml. glass-stoppered Erlenmeyer flask, and add 6 to 10 drops of chlorine-water. Add 3 to 5 drops of potassium cyanide solution (half the volume of chlorine-water), 8 to 10 drops of hydrochloric acid, 1 drop of starch solution, and 3 drops of potassium iodide solution, and titrate with 0·002 N thiosulphate.

Make a blank determination on the reagents.

Results are accurate to within ±1 per cent.

Hypobromite method—To the neutral solution containing up to 10 µg. of iodide, add 3 to 4 drops of sodium hypobromite solution, prepared by treating 20 ml. of 5 per cent. sodium hydroxide solution with 10 ml. of freshly saturated bromine-water. After 15 min., add in a jet 0·5 ml. of a 5 per cent. solution of phenol to remove the excess of hypobromite. Shake three or four times with occasional releasing of the stopper and acidify the solution with 5 drops of 10 per cent. hydrochloric acid solution. Add 1 drop of starch solution, 3 drops of freshly prepared, 20 per cent. potassium iodide solution, and titrate as above, after 5 min.

For 10 to 150 µg. of iodide, use a volume of 8 to 10 ml., 10 drops of hypobromite solution, 1 ml. of phenol solution, 10 to 12 drops of hydrochloric acid, 1 drop of starch, 3 drops of potassium iodide solution, and 0·002 N thiosulphate.

The accuracy is ±1 per cent., a blank determination being necessary on the reagents.

Micro-determination of bromide—Neutralise the solution (1·5 to 20 µg. of bromide in 3 to 5 ml.) to methyl red, add 2 to 3 drops of freshly saturated chlorine-water, and shake the mixture. Add 1 drop of freshly prepared, 5 per cent. potassium cyanide solution and shake thoroughly to convert the bromine chloride and the excess of chloride into bromine cyanide and chlorine cyanide and chloride, loosen the stopper occasionally. Add 4 to 5 drops of hydrochloric acid solution, 2 drops of freshly prepared, 20 per cent. potassium iodide solution, and 1 drop of starch solution. After 10 min. titrate as above, but carefully, as the reaction between bromine cyanide and potassium iodide is slow.

20 to 250 µg. of bromide should be contained in 8 to 10 ml., 8 to 15 drops of chlorine-water and half this volume of potassium cyanide solution, 10 to 15 drops of hydrochloric acid, 3 to 4 drops of potassium iodide solution, 2 drops of starch solution should be added, and the titration made with 0·002 N thiosulphate.

The accuracy is to within −9 per cent. on 1·5 to

3·5 μg., and ±1 per cent. on 16 to 240 μg. Iodide must be absent.

Small quantities of bromide and iodide together—Iodide must be between 10 and 150 μg. and bromide between 16 and 240 μg. Determine the sum by the chlorine method, and then iodide according to the hypobromite method; calculate bromide by difference.

The results for bromine are correct to within −3·5 per cent., and for iodine, to within ±1·85 per cent. M. E. Dalziel

Volumetric Determination of Small Amounts of Soluble Sulphates. C. L. Ogg, C. O. Willits, and F. J. Cooper (*Anal. Chem.*, 1948, **20**, 83–85)—The end-point of the titration of sulphate with barium chloride in presence of tetrahydroxyquinone disodium salt, dipotassium rhodizonate, or disodium rhodizonate is uncertain owing to the local formation of the red barium salt, which reacts only slowly. An apparatus and titration technique which identify the end-point colour and permit continuous comparison of the solution with a standard colour filter are described.

The apparatus consists of a rectangular titration vessel (25- × 45- × 50-mm. high optical absorption cell), a standard 25- × 45-mm. glass colour filter, two 5-ml. burettes graduated to 0·01 ml., and a titration stand. The titration vessel and the light filter are mounted side by side on an opal glass plate. Illumination, preferably fluorescent, is from below and all opal glass is masked except that covered by the titration vessel and the filter. Results are best when no overhead artificial illumination is used.

Procedure—Adjust the solution to pH 6·5 to 7·5, first boiling the acidified solution to expel carbon dioxide if the carbonate content is high. Transfer the solution to the titration vessel and adjust the volume to about 15 ml. Add about 0·08 g. of the indicator and dilute the solution with an equal volume of 95 per cent. ethanol. Titrate with 0·02 *N* barium chloride solution until a permanent colour that matches the colour filter is obtained. Continue stirring for 1 or 2 min. at the end-point to ensure that the colour does not fade, and use a rubber-tipped stirrer to prevent scratching the bottom of the cell. The end-point is taken when an added drop of barium chloride causes a deeper coloration than the colour filter.

Standardise the barium chloride solution against potassium sulphate solution (1·8140 g. per litre), so avoiding the correction factor used when standardisation is made gravimetrically. The titre should not be smaller than 3 ml. in the determinations or in the standardisation, otherwise the apparent normality varies. In the analysis of microsamples, add a known amount of standard potassium sulphate, if necessary, to give a titre greater than 3 ml. The addition can be measured more accurately than the error incurred by a smaller titre.

The standard colour filters of polished glass with 37 per cent. transmission at 550 ± 2 mμ. need not be identical, but the same filter must be used for standardisation and determination. Different turbidities due to the formation of different amounts of barium sulphate in titrations can be compensated for by covering the colour filter by one of a series of microscope slides ground, by means of an aqueous suspension of carborundum, to produce different amounts of diffused light.

Titrations of equal aliquots of a sulphate solution are reproducible to within ±0·02 ml. of the mean value; the average deviation is 0·003 mg. on 1 mg. of sulphur. M. E. Dalziel

Determination of Calcium and Magnesium in Iron Ore by Means of a Cation Exchanger. Yu. I. Usatenko and O. V. Datsenko (*Zavod. Lab.*, 1948, **14**, 1323–1327)—The separation of iron before the determination of calcium and magnesium in iron ores causes difficulties because re-precipitation of ferric hydroxide precipitated by aqueous ammonia solution is essential to avoid loss of calcium and magnesium even when the adsorptive properties of the precipitate are reduced by using concentrated solutions. The use of a cation exchanger (Wofatit R) to absorb calcium and magnesium from solutions containing iron and aluminium in presence of tartaric acid gives a simple and satisfactory separation. [The Wofatit series of ionic exchange resins are referred to in a recent review by Duncan and Lister, *Quarterly Reviews, Chem. Soc.*, 1948, **2**, 307.—G. S. S.]

Preparation of Wofatit filter—To remove cations, Wofatit R was treated five times with concentrated hydrochloric acid at 70° to 80° C. over a total period of 4 hr., and then washed to remove chloride ions. The product gave 0·54 per cent. of ash. About 60 to 65 ml. of the moist Wofatit were placed in a 100-ml. burette over a layer of glass wool.

Tests for absorption—30 ml. of neutral calcium chloride solution, containing the equivalent of 0·0440 g. of calcium oxide, *i.e.*, an amount equivalent to 8·8 per cent. in 0·5 g. of iron ore, were passed through the filter at the rate of 5 ml. per min. The once-filtered solution contained no calcium ions. Similar amounts of calcium chloride solution were treated with 1, 2, 3, 5, and 10 ml. of concentrated hydrochloric acid, each solution was diluted to 50 ml., and passed once through a Wofatit filter, followed by 3 or 4 washes with water, and the filtrates analysed for calcium. With 1 and 2 ml. of acid no calcium was left in solution, but with the other amounts 1·0, 2·5, and 31·0 mg. respectively of calcium oxide were found in the filtrate. Reduction of acid concentration before filtration could not be effected by addition of ammonia, since ammonium ions are absorbed by Wofatit R and the filter then allows calcium ions to pass through.

Mixtures of calcium salts and iron salts in presence of tartaric or citric acid were also passed through Wofatit R filters. All the iron passed through and all the calcium was absorbed. Tartaric acid solution, which often contains calcium as impurity, was purified before use by passing it through a Wofatit filter. Citric acid was less suitable than tartaric acid, being more difficult to wash out of the absorbent. Similar results were obtained with aluminium in place of iron, and with magnesium in place of calcium.

With a mixture of 0·0440 g. of calcium oxide, 0·0320 g. of magnesium oxide, 1 ml. of concentrated hydrochloric acid, and 50 ml. of water, one filtration left 0·2 to 0·5 mg. of calcium oxide and 0·3 to 0·7 mg. of magnesium oxide in the filtrate. In presence of tartaric acid and iron, the amounts in the filtrates increased to 2·2 and 2·8 mg. respectively. Second filtrations, however, gave complete absorption.

Extraction—When a used filter was treated with 25 ml. of concentrated hydrochloric acid, introduced in small portions, and then washed 3 or 4 times with water, only 33·2 mg. of calcium oxide instead of 44·0 mg. were extracted. After two further treatments with 15-ml. portions of acid, the total extracted was still only 42·8 mg. Treatment with 100 ml. of diluted hydrochloric acid (1 + 4) gave complete extraction of calcium oxide and magnesium oxide in four operations.

Procedure—Place 0·5 g. of the finely divided ore in a 100-ml. beaker, moisten with water, and heat under cover with 15 ml. of concentrated hydrochloric acid until there are no dark coloured particles on the bottom of the beaker. Remove the cover and evaporate the solution to a syrup, add about 30 ml. of hot diluted hydrochloric acid (1 + 4), stir, filter, wash the residue with acidified water until the washings are no longer yellow, evaporate the filtrate to 2 to 3 ml., dilute to 30 ml. with water, and add 20 ml. of 20 per cent. tartaric acid solution. Run the solution at the rate of 5 ml. per min. through a Wofatit R filter, test the filtrate for absence of calcium by means of ammonium oxalate solution, wash the filter 3 or 4 times with water and reject the washings, treat the washed filter with four 25-ml. portions of diluted hydrochloric acid (1 + 4), and then wash 3 or 4 times with water. In the filtrate determine calcium and magnesium by the usual gravimetric or volumetric methods.

With contents of about 1 per cent., the results for percentages of calcium or magnesium oxide differed from the mean by up to 5 parts in 100, but with higher contents (up to 6·7 per cent.) the variation was only about ±1 part in 100.

G. S. Smith

Removal of Manganese in the Determinations of the Zinc, Calcium, and Magnesium of Manganese Ores and Products. R. L. Evans (*Anal. Chem.*, 1948, **20**, 87)—The standard procedure (Hillebrand and Lundell, *Applied Inorganic Analysis*, John Wiley and Sons, 1929) is modified. After adding potassium perchlorate, evaporate the suspension cautiously to dryness on a low-temperature hot-plate or steam-bath; bumping is avoided by heating below the boiling-point. Moisten the residue with a few millilitres of nitric acid and digest in 50 to 100 ml. of cold water. Filter with or without suction, and wash the beaker and residue with an equal amount of water. The combined filtrate and washings then have a suitable nitrate concentration for the direct determination of zinc, calcium, and magnesium without a further evaporation.

With good filtration the method removes up to 1 g. of manganese leaving a clear solution of manganese content equivalent to less than 3 drops of 0·1 *N* potassium permanganate. Re-precipitation of manganese from the combined residues of six samples gave a filtrate containing less than 1 mg. of calcium or magnesium.

M. E. Dalziel

Spectrographic Determination of Beryllium in Light Industrial Alloys. A. C. Pruig (*Informacion Quim. Analit.*, 1946, **1**, 21–23)—A rapid method for determining beryllium in alloys rich in magnesium by means of a spark discharge between suitable electrodes is described.

Method—Dissolve 0·165, 0·330, and 0·660 g. of trihydrated beryllium nitrate by warming in 50-ml. portions of hydrochloric acid. Cool the solutions to room temperature and dilute each one to 100 ml. with water. Prepare three solutions each containing 1 g. of beryllium-free Electron alloy dissolved in 40 ml. of diluted hydrochloric acid (1 : 1). Dilute each solution to 100 ml. with water and add to each 1 ml. of the beryllium solution; mix well.

Use a regular discharge from a Feussner generator between high-purity carbon electrodes prepared by Gatterer's method, and the following excitation conditions: FF4, C1/5, L1/1, I 0·75 amp., distance between electrodes 2 mm., standard electrodes of 5 mm. diameter, simple type diaphragm (Zeiss Spectrograph Q–24), focus 3031 A., aperture 0·03 mm., and time of preliminary discharge 1 min. between the electrodes before the test solution is added.

Place 2 drops of test solution on each electrode by means of a thin platinum thread, 0·2 mm. in diameter, having one end bent into a circle 3 mm. in diameter. Allow the electrodes to dry for 1 min., pass a spark discharge for 1 min., the optimum time for small concentrations of beryllium. Use Agfa Phototechnicher Film B, a developer containing Metol 2 g., hydroquinone 4 g., sodium sulphate 25 g., sodium carbonate 18·5 g., potassium bromide 2 g., and water up to 100 ml., a development time of 4 min. at 18° C., and a fixing solution containing 400 g. of sodium thiosulphate, 100 ml. of sodium hydrogen sulphite (38° Bé), and water up to 1 litre.

Use a microphotometer to measure the difference in photographic blackening of the lines Be 3130·4 and Mg 2915·5, and plot the logarithms of the concentrations of beryllium against the logarithms of the ratios of intensities of these lines.

The graph obtained can be used to standardise electrodes made from Electron alloy containing beryllium, and these can, in turn, be used as standards of comparison for beryllium alloys, the necessity of preparing special electrodes by fusion of the component elements thus being eliminated.

A. F. Sturgess

Volumetric Micro-determination of Arsenic and Iron. G. F. Smith and J. S. Fritz (*Anal. Chem.*, 1948, **20**, 874–876)—The volumetric micro-determination of oxalate by titration with perchloratoceric acid in perchloric acid solution, 5-nitro-1 : 10-phenantholine ferrous complex being used as indicator, has been extended to include the micro-determination of iron and of arsenic.

Standardisation of 0·001 *N* perchloratoceric acid in 2 *M* perchloric acid against sodium oxalate in 2 *M* perchloric acid is effected according to the method of Salomon, Gabrio, and Smith (*Arch. Biochem.*, 1946, **11**, 433; *Analyst*, 1947, **72**, 310) for calcium except for the use of weight burettes for sampling. The end-point is marked by a red to faint blue colour change of the indicator. Similar titration of a known arsenite solution gave values ranging from 0·0042 mg. low to 0·0007 mg. high on samples containing from 0·270 to 0·153 mg. of arsenic trioxide. On a known iron solution, reduced by passage through a Jones micro-reductor, the values were from 0·0007 mg. low to 0·0022 mg. high on samples containing from 0·197 to 0·314 mg. of iron.

The values for arsenic are predominantly low, and for iron, predominantly high. M. E. DALZIEL

Determination of Mercury in Organic and Inorganic Compounds. Stannous Chloride Reduction Method. J. N. Bartlett and W. M. McNabb (*Anal. Chem.*, 1947, **19**, 484–487)—By modifying the Krieckhaus method (Low, *Technical Methods of Ore Analysis*, John Wiley and Sons, 1919, p. 181), errors due to loss of mercury during digestion and to incomplete washing of the free mercury are reduced, and the range of useful accuracy is increased to between 0·03 and 0·3 g. of mercury.

Inorganic mercury—Treat 50 ml. of solution containing about 100 mg. of mercury with 10 ml. of concentrated hydrochloric acid and 10 ml. of stannous chloride solution in a 125-ml. Erlenmeyer flask. Prepare the stannous chloride solution by dissolving 125 g. of stannous chloride dihydrate in 70 ml. of concentrated hydrochloric acid and diluting to 250 ml., the solution being heated over tin until clear and then stored over tin. Insert a "cold finger" into the flask to about 2 cm. above the liquid surface and heat the solution until the mercury forms into globules. Filter through a Gooch crucible and wash the metal by decantation with 2 *N* sulphuric acid and water until freed from chloride ions. Transfer the mercury and the asbestos mat to a 125-ml. Erlenmeyer flask and by means of 5 ml. of hot, concentrated nitric acid dissolve the finely divided mercury remaining on the walls of the crucible. Collect the acid in the flask and dissolve the remainder of the mercury. Dilute the solution to 35 ml., add dropwise a slight excess of saturated potassium permanganate solution, and destroy the excess by means of a 10 per cent. solution of ferrous sulphate in 0·3 *N* sulphuric acid. Cool the solution to about 15° C., add 0·4 ml. of ferric alum indicator, and titrate with standard potassium thiocyanate.

The results given are all low but correct to within 1 part in 330.

Procedure for determining semi-micro amounts of mercury—Weigh a sample containing between 30 and 100 mg. of mercury into a 15-ml. centrifuge tube and add 5 ml. of water and 2 or 3 drops of hydrochloric acid. Heat to 50° C. and add a mixture of 3 ml. of concentrated hydrochloric acid and 3 ml. of stannous chloride solution, also at 50° C. Allow to stand for 5 min., re-heat to 50° C., and centrifuge for 5 min. at 1500 r.p.m. Repeat the heating and centrifuging if the mercury is still not globular. Remove the supernatant liquid by means of a fine-porosity filter-stick of about 10-mm. diameter. Wash the mercury three or four times with 2-ml. portions of 2 *N* sulphuric acid and then with water until free from chloride, removing the wash-liquids by means of the filter-stick. Disconnect the filter and place it in the centrifuge tube with 2 ml. of concentrated nitric acid for 3 to 4 min. to dissolve the mercury, and transfer the solution to a 125-ml. Erlenmeyer flask; wash the filter by drawing water through it in the reverse direction, and collect the washings in the flask. Add potassium permanganate, dilute, and titrate as in the macro-method.

Results of 14 analyses of 5 compounds gave values ranging from 1 part in 500 low to 1 part in 300 high, 10 of the values being low.

Organic mercury—Precipitation of mercury without preliminary oxidation of the organic matter necessitates the use of a water-soluble solvent so that addition of the reducing mixture does not precipitate the compound itself. Acetone, ethanol, glacial acetic acid, dioxan, and hydrochloric acid were tried.

Procedure—Place a sample containing 50 to 70 mg. of mercury in a 200 × 25 mm. Pyrex test tube and add 3 ml. of dioxan containing 2 drops of hydrochloric acid to rinse the sample into the base of the tube. Add a mixture of 2 ml. of stannous chloride solution and 2 ml. of hydrochloric acid, and insert a 180 × 15 mm. cold finger to a point directly above the liquid surface. Lower the test tube into an oil-bath and digest at 115° C. until the mercury becomes globular (25 to 30 min.). Add 2 ml. of water and if the liquid remains clear for 3 min., the reduction may be regarded as complete, otherwise digest for a further period. Remove and rinse the cold finger, and filter and wash the mercury as before. Add 2 ml. of concentrated nitric acid to the test tube and transfer the resulting solution to a 125-ml. Erlenmeyer flask, rinsing the crucible with 2 ml. of hot nitric acid; dilute the solution and complete the determination as for inorganic compounds.

Twenty-five values were obtained for 8 compounds ranging in mercury content from 23 to 64 per cent.; and the values for any one compound agreed to within 1 part in 250; the maximum deviation of a mean value from the value obtained by the method of Sloviter *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 890–893) or of Rauscher (*Ibid.*, 1938, **10**, 331–333) was 1 part in 320. M. E. DALZIEL

Determination of Copper in Steels by Colorimetric Titration with Dithizone. I. V. Suprunovich and A. B. Konovalova (*Zavod. Lab.*, 1948, **14**, 1061–1063)—From a solution containing citric acid and disodium hydrogen phosphate dithizone extracts copper, platinum, palladium, gold, silver, mercury, bismuth, and stannous tin. Of these elements platinum, palladium, and gold are not found in steels, tin can be oxidised to the stannic form, which does not interfere, and the other elements give dithizonates that are decomposed by shaking the extract with potassium iodide solution. The oxidising action of ferric iron is prevented by its combination in a complex. Copper can be determined by colorimetric titration of the extract, the mixed colour of the dithizonate and free dithizone being used. The mixed colour gives more accurate results than that of the pure dithizonate since in the latter case the excess of reagent may be incompletely extracted or the dithizonate may be partly decomposed; and, furthermore, the sharpest colour change is obtained with a 50 per cent. excess of the reagent present. The colour is a mixture of reddish-violet and green giving a violet or grey.

Reagents—Buffer solution—Dissolve 38 g. of citric acid and 21 g. of disodium hydrogen phosphate in water, shake with a concentrated solution of dithizone in carbon tetrachloride, run off the lower layer, wash the aqueous solution with carbon tetrachloride, and dilute with twice-distilled water to 250 ml. *Potassium iodide solution*—Dissolve 10 g. of potassium iodide in 450 ml. of water acidified with 5 ml. of *N* hydrochloric acid, shake with dithizone solution as with the buffer solution, and dilute to 500 ml. *Standard copper solution*—Dissolve 0·5 g. of electrolytic copper in 20 ml. of 6 *N* nitric acid, evaporate nearly to dryness, add 2 or 3 drops of glacial acetic acid, and dilute to 500 ml. in a graduated flask. Before use dilute a portion to give a solution containing 1 μg. in 1 ml.

Procedure—Dissolve a suitable weight of steel in 100 ml. of diluted sulphuric acid solution (1 + 9), and add nitric acid dropwise to the boiling solution until the colour becomes light yellow. Cool, dilute to 250 ml., and place an aliquot portion containing 10 to 20 μg. of copper in a 150-ml. separating funnel. Dilute to 25 ml. with 10 per cent. sulphuric acid solution, add 2 drops of 0·02 per cent. cresol red solution, add aqueous ammonia carefully until the colour changes from red to yellow, and add 2 ml. of the buffer solution. Then add 4- or 5-ml. portions of a solution of 15 mg. of dithizone in 100 ml. of carbon tetrachloride, shaking after each addition for 2 to 3 min., and pouring the extract into a 50-ml. graduated colorimeter tube. Continue the extractions until the last is green, and add to the solution in the tube a volume of dithizone solution equal to about half that used in the extractions. Shake the contents of the tube for 2 min. with 10 ml. of the acidified potassium iodide solution. In another 50-ml. tube place a similar volume of dithizone solution, add a similar amount of sulphuric acid and cresol red, neutralise with aqueous ammonia, add the buffer solution, and then run in the standard copper solution with shaking until the colours match.

The determination takes 25 to 30 min. The method was tested on copper-free steels with added copper, on alloy steels containing chromium, nickel, and molybdenum, and on cobalt steels. Errors with copper contents of 0·1 to 0·2 per cent. are insignificant. G. S. SMITH

Polarographic Determination of Copper and Nickel in Steel. A. G. Stromberg, R. V. Dityatkovskaya, and N. V. Milovanova (*Zavod. Lab.*, 1948, **14**, 919–925)—Adsorption of copper and nickel on ferric hydroxide precipitated by ammonia renders many published methods for the polarographic determination of copper and nickel inaccurate. Experiments to test the degree of adsorption in 50 ml. of a solution containing 0·5 g. of iron in presence of various concentrations of ammonium chloride and ammonia have now shown that when the ammonium chloride concentration is 0·7 *N* the adsorption of copper becomes nil at ammonia concentrations of not less than 2·5 *N*, and of nickel at concentrations of not less than 3·0 *N*.

Copper and nickel in steel—Procedure—Dissolve 0·5 g. of steel in 25 ml. of diluted hydrochloric acid (1 + 1) with 1 to 2 ml. of concentrated nitric acid, evaporate to dryness, add 4 ml. of diluted hydrochloric acid (1 + 1) and 5 ml. of water, and heat to dissolve the residue. Cool, transfer to a 50-ml. graduated flask, add 15 ml. of concentrated aqueous ammonia, dilute to the mark, mix, and leave for 10 to 15 min. for the precipitate to settle. Pipette an aliquot portion of the clear solution into a polarographic cell, add to it 4 ml. of saturated sodium sulphite solution and 1 ml. of 0·25 per cent. gelatin solution, mix, and obtain the polarogram between −0·4 and −1·2 v. Measure the separate copper and nickel waves.

Comparison of results with those of chemical determinations showed that differences did not exceed 0·02 per cent. with copper and nickel contents of 0·1 to 0·5 per cent. With nickel between 1 and 4 per cent., the differences for nickel were between 0·03 and 0·10 per cent. G. S. SMITH

Determination of Phosphorus Pentoxide in Phosphate Rock. J. L. Kassner, H. P. Crammer, and M. A. Ozier (*Anal. Chem.*, 1948, **20**, 1052–1055)—When ammonium molybdiphosphate is determined volumetrically by dissolution in an excess of standard sodium hydroxide solution and back-titration of the excess of alkali with phenolphthalein as indicator, the results obtained for phosphorus pentoxide are 0·3 per cent. higher than by the normal gravimetric determination as magnesium pyrophosphate. The alkalimetric method is improved by using a mixed indicator that changes colour at the stoicheiometric end-point, and by precipitation of the ammonium molybdiphosphate under conditions that ensure a uniform composition of the product.

Reagents—Indicator—Dissolve 0·1000 g. of phenol red and of bromothymol blue in an excess of sodium hydroxide solution, adjust the pH of each solution to 7·5 with nitric acid, and dilute each accurately to 250 ml. Mix 40·0 ml. of the bromothymol blue solution and 25·0 ml. of the phenol red solution for the indicator and use 0·5 ml. for each 100 ml. of solution at the end-point.

Citromolybdate solution—Dissolve in 1360 ml. of water, without heating, 54 g. of ammonium nitrate, 52·6 g. of citric acid, and 68 g. of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, and pour the solution into a mixture of 253 ml. of concentrated nitric acid (sp.gr. 1·42) and 310 ml. of water. Clarify the solution by adding filter paper pulp or a few drops of diammonium hydrogen phosphate solution and boiling for 5 to 10 min.; allow to stand overnight before siphoning off the clear solution.

Procedure—Treat 1 g. of the sample with 15 ml. of water, and add 5 ml. of concentrated nitric acid and 10 to 20 ml. of 60 to 70 per cent. perchloric acid; heat to copious fuming. Cover the container and boil the solution gently for 20 min. to dehydrate the silica. Allow to cool somewhat, add 75 ml. of water, and heat to boiling; then filter the solution into a 250-ml. flask, and wash the filter with hot dilute nitric acid and hot water. Treat the silica with hydrofluoric acid and nitric acid and fuse the residue with sodium carbonate; transfer the extracted melt to the bulk of the solution to ensure complete recovery of the phosphate. Dilute the solution to 250 ml.

Add 80 ml. of the citromolybdate solution to a 25-ml. portion of the solution, heat to boiling, and maintain at this temperature for 2 to 3 min. before filtering either hot or cold. Wash the precipitate 4 or 5 times by decantation with 20-ml. portions of neutral, cold, 1 per cent. potassium nitrate solution or water, and transfer it to a filter crucible and wash ten times more. Place the crucible in the original beaker and dissolve the precipitate in an excess of about 15 ml. of 0·3 *N* sodium hydroxide (carbonate-free). Add 1 ml. of the mixed indicator solution and titrate the solution with 0·1 *N* nitric acid until yellow. Remove the crucible and wash it with water. Adjust the volume of the solution to 200 ml., and titrate back the excess of acid with 0·3 *N* sodium hydroxide to a permanent light purple colour, viewed by direct sunlight or a fluorescent daylight lamp.

Results—The maximum deviation in 30 consecutive samples was 0·1 mg. of phosphorus pentoxide.

M. E. DALZIEL

Spark Technique in Spectrographic Analysis of Slags. R. H. Steinberg and H. J. Belic (*Anal. Chem.*, 1948, **20**, 72–73)—A rapid spectrographic method by which the lime/silica ratio of open-hearth steel-furnace slag can be estimated is given. With the spark technique used an extremely high vapour temperature is obtained and the electrodes are comparatively cool, hence calibration curves are stable.

METHOD—*Apparatus*—The spark source is a commercial 2-kVA high-voltage spark unit with an added inductance of 0·045 millihenry and a capacitance of 0·021 microfarad. The unit has a Fuessner synchronous rotating gap. The spectrograph used is a 1·5-m. instrument with a 24,000 lines per inch grating providing a dispersion of 7 A. per mm. A graphite electrode 0·25 in. in diameter cut flat at one end and with a hole 0·1 in. in diameter and 0·125 in. deep cut in the flat surface is used.

Procedure—Pack the cup of the electrode by pressing the electrode into a pile of the powdered slag. Add a drop or two of a 2 per cent. solution of Ethocel (ethyl cellulose ether) in butyl acetate to the slag to lengthen the time of "blow away." Other organic cementing compounds may be satisfactory. Remove the solvent by aeration or heating. Use a graphite rod of the same size but with a cone-shaped tip as the other electrode and spark for about 10 sec. After developing the film by standard technique, use a microphotometer to measure the density of the relevant lines on the film. For lime/silica ratios from 0·6 to 3·0 calculate the logarithm of the relative intensity of the 3905·53 A. silicon and the 4302·53 A. calcium lines, and for lime/silica ratios from 2·5 to 8·5 compare the 3905·53 A. silicon line with the 4318·65 A. calcium line. From analyses of standard samples prepare a graph of the logarithms of the lime/silica ratios against the logarithms of the relative intensities for each of the pairs of lines.

The reproducibility of results is approximately to within 10 per cent. If free lime is present, the figure for the lime/silica ratio is high.

B. ATKINSON

Physical Methods, Apparatus, etc

Crystallographic Data. Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 1124–1125)—Crystal data for 1 : 3 : 5-tri-(*p*-chlorophenyl)-benzene are given under the titles proposed in the original paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, 73, 579).

M. E. DALZIEL

Crystallographic Data. Armour Research Foundation of the Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 1249–1250)—2-Methylnaphthalene is described under the headings listed in the original paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579).

M. E. DALZIEL

Reviews

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Vol. IX: Oils, fatty—Pituitary Body. Pp. 671. London: Longmans, Green & Co., Ltd. 1949. Price 80s.

Perhaps one does not take down a Dictionary of Applied Chemistry from the shelf for entertainment or general reading, yet one may do so with this volume of "Thorpe." It is quite fascinating. If one reads on there is a chance of getting a sort of mental indigestion as the change from, say, the phase rule to photosynthesis is rather great, but it's all very readable and absorbingly interesting. Moreover, this volume seems to contain major articles one or more of which must be of interest to almost any variety of chemist. Who is not interested either in oils and fats, oxidising enzymes, petroleum, phosphorus, pigments, photography, particle size or photosynthesis? And he could browse on pituitary body or orthobaric density just as a *hors d'oeuvre*, and then turn to pest control for "afters"; opium is there if he needs it, and by way of liqueur, there is perillyl alcohol.

The aim of the Dictionary is to provide a balanced treatment of modern chemistry, including both original work and industrial aspects. It must be said that the editors and contributors have done this task very well indeed in the present volume and the index adds to its utility. One could, of course, comment on the inclusion of this or the exclusion of that, but the outstanding fact is that it is most useful and contains just that information and those references that the busy chemist, specially the analyst and consultant, will most need. It is difficult to compare one article with another. The analysis of fatty oils is useful and informative; photosynthesis or pest control are inevitably more interesting as well as being equally useful.

Petroleum provides a major article, almost a book in itself, which is full of interest as well as of utility. So one might go on. The fact is that the whole volume is excellent and one that every chemist must either possess or at least have access to. Besides those major articles already mentioned, perhaps one should draw attention to paper-making (S. F. Edge), Vitamin P (Bergel), penicillin, patulin, oxalic acid, phthalic acid, oxazines and oxygen as further examples of the matter available in this ninth volume of the best chemical dictionary in the English language.

The answer to anyone reading a review to help him to decide if he should buy the book is—of course you must, it is excellent. H. E. Cox

PRACTICAL METHODS IN BIOCHEMISTRY. By F. C. KOCH and M. E. HANKE. Fifth Edition. Pp. ix + 419. Baltimore: The Williams & Wilkins Co. London: Bailliere, Tindall & Cox. 1948. Price 16s. 6d.

The precise nature and scope of biochemistry is still somewhat of a mystery to chemists working in non-biological fields, and many still apparently think of it as the type of chemistry that deals—to paraphrase a famous saying—with blood, sweat and urine. The book now under review appears to give colour to this limited concept, for it deals exclusively with that branch of the subject known as physiological or clinical biochemistry. In this respect it is an excellent student's manual, and he who has worked through the 283 experiments described in it can reckon himself to be a competent physiological biochemist. But there are other aspects of biochemistry not covered by this book—a biochemistry of nutrition, of therapeutics, of micro-organisms and plants—and this ought surely to be made clear by authors who claim to write textbooks of biochemistry.

Considered as a textbook of practical methods used in clinical biochemistry, the book could hardly be bettered. The first part covers the chemistry of cell constituents and the five chapters comprising it deal respectively with carbohydrates, fats, proteins and amino acids, nucleoproteins and nucleic acids, and hydrogen ion activity and pH. The second part is on the chemistry of the digestive tract and the third, the longest of the three, is on blood and urine. Every conceivable test of importance in clinical diagnosis appears to be referred to, and several of Van Slyke's manometric methods are given in detail, together with instructions for using the apparatus and the requisite tables for calculating the results. A chapter on colorimetric and fluorimetric methods of estimating vitamins and another on microbiological assays are included for the first time in this edition; the instructions are precise, useful and up-to-date.

The book is intended primarily as a practical companion to a particular American textbook of theoretical biochemistry, but it can obviously be used independently. One is left wondering, however, to what extent it is good for a student to be given such detailed instructions. Would it not be more profitable for him if he were made to search through the original literature for the methods to be used—at all events in the later part of his course?

This very objection, however, makes the book of value to others, and many analysts, especially those employed in hospital laboratories, would find this book useful, since it collects together in a handy form most of the standard methods he is likely to need.

The only criticism that may perhaps be offered is that some of these methods have not been brought up-to-date, for some of the references given are comparatively old, and for most methods, however well tried, refinements have been introduced in recent years. Surely these ought to be taken note of in a detailed textbook of analytical methods. Apart from this one point, however, the book has few blemishes; it is singularly free from printer's errors, it is well printed and bound, it has a good index and its price is moderate.

F. A. ROBINSON

ANALAR STANDARDS FOR LABORATORY CHEMICALS. The British Drug Houses Ltd. and Hopkin & Williams Ltd. Fourth Edition. Pp. xviii + 302. London. 1949.

The object of the publication of this latest edition of AnalaR Standards is to provide chemists with a revised and up-to-date series of specifications for laboratory chemicals; apart from this object it will be found of considerable value in the application of its assays and methods of test to other analytical problems. It has been considerably revised and enlarged with specifications for 58 new "AnalaR" chemicals; compounds of ten elements not previously in the range is a feature of the additions. In order to obtain the correct interpretation of the significance of the impurity tests, the user of the book should be familiar with the introductory explanatory notes.

The introduction in this edition of newer methods such as the determination of water by the Karl Fischer technique, electrolytic deposition and polarographic methods, breaks away from the classical standardisation necessary in more authoritative works; the polarograph is particularly adaptable to estimation of small amounts of contaminants in "pure" chemicals. The compilers of the book point out that introduction of newer methods of great sensitivity may show that a figure previously quoted as the maximum limit of an impurity has had to be revised, but that the purity of the product has not, in fact, been lowered.

Very little criticism of the book can be made; one minor editorial point might be mentioned of the unnecessary selection of arsenic for transcribing the maximum impurity limit from percentage to parts per million in each monograph.

The book is very well produced, the print is clear and the binding robust, and no printing errors were detected. It is very useful and this new edition should find a place on the working bench shelf of every laboratory.

D. C. GARRATT

Erratum: August (1949) issue, p. 479.

In the 7th line below the figure, *for* "between the two slides," *read* "between the two solids".

THE DETERMINATION OF RIBOFLAVINE BY CHEMICAL MEANS

THE attention of analysts is drawn to the following communication which has been received from the Sub-Committee on Vitamin Estimations of the Analytical Methods Committee:

The Chemical Panel of the Sub-Committee on Vitamin Estimations of the Analytical Methods Committee was asked to investigate existing chemical methods for the determination of riboflavine and to report whether any of them or a modification of any of them could be accepted as reliable for a range of foodstuffs embodying cereals, yeast and yeast products, meat products and malt products.

This Panel has made a full investigation of the problem; it has come to the conclusion that, although one modified technique shows distinct promise, none of the methods studied can at present be recommended to the practising analyst as reliable for so wide a range of foodstuffs. The formulation of a technique capable of general application even in the defined field appears to depend upon the development of a fluorimeter of better performance than those at present available, in order to secure increased sensitivity and a greater degree of reproducibility in results, particularly between one observer and another.

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Applications of the Intermittent A.C. Arc. Part II. Determination of Rare Earths

By J. A. C. McCLELLAND

(Paper presented at the Joint Meeting of the North of England Section and the Physical Methods Group at Liverpool, on October 2nd, 1948)

The chemical separation and quantitative determination of rare earth elements is a laborious procedure, and when the need arose for analysis of complex minerals for these elements, a spectrographic finish was obviously desirable provided that adequate sensitivity and precision could be obtained.

One factor affecting sensitivity must be the interference by substances other than those to be determined, and this effect may be minimised by separating the desired elements from the major constituents of the sample.[1]

Chemical extraction of the rare earths, as distinct from their individual separation, is a comparatively simple process and by this means very small amounts may be determined, depending only on the quantity of sample available. Such a process has been used.

Accuracy as well as ultimate sensitivity will depend largely on the means of excitation used. The intermittent A.C. arc[2,3] has been found to give a good sensitivity and reproducibility in this determination. A precision of the order of ±10 per cent. of the proportion of the element being determined was obtained, using prepared mixtures of pure rare earth oxides with iron oxide.

Some difficulty was at first experienced in obtaining by fusion a consistently smooth and even coating on the electrode. Investigation showed this failure to be associated with the particle size of the iron oxide; a remedy was found in the control of conditions of precipitation and ignition.

EXPERIMENTAL

(*a*) PRELIMINARY CHEMICAL CONCENTRATION—

For spectrographic purposes the essential point in any chemical separation of the rare earths is to obtain by the simplest means a concentration of rare earths substantially free from all other elements that might cause interference in the spectrographic technique. The amount of sample used for the determination will be governed by the amount of rare earth it is suspected of containing, and it will be necessary at the outset to take the sample to complete solution. When the material is a silicate rock, prolonged treatment with hydrofluoric acid will be necessary before any separation of the rare earths can be accomplished. Materials of this nature usually contain large amounts of other elements, such as iron and aluminium, from which the rare earths must be separated.

If appreciable quantities of uranium salts are present the method described by Short and Dutton[4] or by Hirt and Nachtrieb[5] may be used. The former describe a process which separates rare earths from large amounts of uranium salts, involving precipitation of the rare earths as fluorides and hydroxides, thereby avoiding the less satisfactory separation of the rare earths as oxalates.

The separation of rare earths from uranium salts has not, however, arisen in the present work, and a simpler method of precipitation has been used, an iron salt being added to serve as an internal standard.

Taking 1 g. of the original sample from which the rare earths are to be concentrated and adding 0·25 g. of iron oxide to the rare earths obtained, the following percentages of rare earth oxides may be determined. The figures given are for determination of rare earths by means of the lines given in Table II, which have been selected, where possible, with emphasis on precision rather than sensitivity; therefore these ranges do not necessarily record the lowest possible limit of detectability—

Y	La	Ce	Pr	Nd
0·0005–0·025%	0·001–0·025%	0·005–0·05%	0·002–0·05%	0·002–0·05%
Sm	Eu	Gd	Tb	Dy
0·002–0·05%	0·0002–0·01%	0·002–0·05%	0·002–0·05%	0·0005–0·02%
Ho	Er	Tm	Yb	Lu
0·0002–0·01%	0·0005–0·01%	0·001–0·025%	0·0002–0·01%	0·0002–0·01%
Sc	Th			
0·0002–0·01%	0·025–0·25%			

The particle size of the iron oxide was found to be important in obtaining consistent results by the fusion technique, and conditions of precipitation and of ignition were adjusted to give the desired results in this respect. Electron micrographs, for which I am indebted to Mr. B. S. Cooper of the Research Laboratories of the General Electric Company Ltd., were taken in an attempt to solve this problem.

In preparing the samples where the amount of iron oxide is relatively small, it is necessary to avoid too fine a particle size. To achieve this the precipitate is washed to the bottom of the filter paper and ignited in as compact a mass as possible.

(*b*) STANDARDS—

For the preparation of standards the iron oxide is prepared from Specpure iron and the final precipitation carried out in presence of filter paper pulp. Subsequent ignition at 800° C. produces an iron oxide that is not too coarse.

The following standards were prepared. Percentages given are based on the oxide mixtures: subsequent dilution with potassium hydrogen sulphate reduces the percentage of rare earth in the actual mixture used on the electrode to one-fifth of these figures.

Ho—1·6, 0·8, 0·4, 0·16, 0·08 and 0·04 per cent.
Ce—3·0, 1·5, 0·75, 0·4 and 0·2 per cent.
Th—10·0, 5·0, 3·3 and 1·0 per cent.
Others—2·0, 1·0, 0·5, 0·2, 0·1, 0·05 and 0·02 per cent.

Note—All the rare earth oxides used were pure except that of holmium which contained 80 per cent. of holmia together with dysprosia and yttria (Johnson, Matthey & Co., spectrographically standardised).

From these single standards, composite standards were prepared: these standard mixtures, as will be seen from Table I, usually included about four different rare earth elements. Owing to the cost of some of the rare earth oxides it was not possible to prepare one standard to embrace all those investigated, although such a standard would be exceedingly useful in routine analysis of this nature.

The sensitivity of thorium in the arc discharge is much lower than that of the other elements investigated in this work, and is not included in the standard mixtures tabulated below. It is, however, of not inconsiderable importance in analysis involving the determination of rare earths.

(*c*) SPECTROGRAPHIC TECHNIQUE—

The concentrate of rare earth oxides in iron oxide, obtained from the preliminary chemical treatment of the sample, such that the ferric oxide content is not substantially less than ten times the total rare earth content, is finely ground with a weight of potassium hydrogen sulphate four times that of the ferric oxide.

Standards are prepared from pure rare earth oxides and pure iron oxide well ground and mixed with potassium hydrogen sulphate so that $KHSO_4 : Fe_2O_3 = 4 : 1$ as before.

TABLE I

STANDARD MIXTURES

Percentage calculated on total oxide before addition of $KHSO_4$

	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Sc
1	0·07	—	1·0	—	—	—	0·07	—	—	—	—	—	—	—	—	—
2	0·12	0·25	0·75	—	—	—	0·12	—	—	—	—	—	—	—	—	—
3	0·25	0·12	0·4	—	—	—	0·25	—	—	—	—	—	—	—	—	—
4	0·33	0·33	—	—	—	—	0·03	—	—	—	—	—	—	—	—	—
5	—	0·5	1·5	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	0·66	0·33	—	—	—	—	—	—	—	0·07	—	—
7	—	—	—	1·0	—	1·0	—	—	—	—	—	—	—	—	—	—
8	—	—	—	0·5	0·12	0·5	—	—	—	—	—	—	—	0·50	—	—
9	—	—	—	0·25	0·25	0·12	—	—	—	—	—	—	—	0·25	—	—
10	—	—	—	0·16	0·33	—	—	—	—	—	—	—	—	0·16	—	—
11	—	—	—	0·5	0·5	0·5	—	—	—	—	—	—	—	0·25	—	—
12	—	0·25	1·5	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	0·165	1·0	—	—	—	—	—	—	—	—	—	—	0·165	—	—
14	—	0·66	0·5	—	—	—	—	—	—	—	—	—	—	0·065	—	—
15	—	0·33	0·25	—	—	—	—	—	—	—	—	—	—	0·33	—	—
16	—	—	—	—	—	—	—	1·0	—	—	—	—	—	—	—	0·25
17	—	—	—	—	—	—	—	0·66	—	0·33	—	—	—	—	—	0·165
18	—	—	—	—	—	—	—	0·33	—	0·165	—	—	—	—	—	0·07
19	—	—	—	—	—	—	—	0·165	—	0·07	—	—	—	—	—	0·03
20	—	0·66	—	0·66	—	—	—	0·165	—	—	—	—	—	—	—	—
21	—	0·5	—	1·0	—	—	—	—	—	—	—	—	—	—	—	—
22	—	0·33	—	0·33	—	—	—	0·66	—	—	—	—	—	—	—	—
23	—	0·17	—	0·17	—	—	—	0·33	—	—	—	—	—	—	—	—
24	—	0·25	—	—	—	—	—	1·0	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	0·4	—	0·15	0·04	0·04	—	0·2	—
26	—	—	—	—	—	—	—	—	0·2	—	0·08	0·1	0·1	—	0·1	—
27	—	—	—	—	—	—	—	—	1·0	—	—	—	—	—	0·05	—
28	—	—	—	—	—	—	—	—	0·66	—	—	0·33	0·33	—	—	—
29	—	—	—	—	—	—	—	—	—	—	0·4	—	0·5	—	—	—
30	—	—	—	—	—	—	—	—	—	—	0·28	0·66	—	—	0·33	—
31	—	—	—	—	—	—	—	—	0·4	—	0·16	0·2	0·2	—	0·2	—
32	—	—	—	—	—	—	—	—	0·66	—	0·28	—	0·33	—	—	—

The resulting powder is placed in a cupped graphite electrode having a crater 1 to 1·5 mm. deep, and levelled off. The electrode is heated on a hot-plate until fusion just occurs, whereupon the melt is spread evenly and rapidly over the surface with a steel blade to give a smooth coating to the electrode.

Pairs of such electrodes are used in the intermittent A.C. arc discharge under the following conditions—

Spectrograph	Large Littrow instrument setting 2900–6000 A.
Source to slit	38 cm. with lens F.1025.
Slit width	1½ divisions, 0·015 mm.
Electrodes	Graphite ¼-inch diameter J.M.4.
Electrode separation	4 mm.
Capacitance	48 μF.
Current	4 amp.
Plate	Ilford Isozenith developed 5½ min. in I.D.2 at 66° F.
Exposure	0/90 sec.

Note—Correct exposures give Fe 4337·0 A. a density of 0·6 to 0·7. The spectrograph on which this work was done was fitted with an old type of slit for which 1 division ≡ 0·01 mm. and it required some preliminary experiments to adjust the slit width with sufficient accuracy to obtain the correct density.

(*d*) LINE PAIRS—

A preliminary survey of the rare earth spectra was made as a guide to the choice of suitable line pairs and to the sensitivity and reproducibility to be expected under the conditions of excitation used. In this, the survey recently made by Smith and Wiggins[5] on the rare earth spectra with special reference to the wavelength and intensity of the most sensitive lines proved very valuable.

Difficulties were encountered in the selection of suitable line pairs, as many of the more sensitive lines are masked by the bands that are produced in the graphite arc. Furthermore, lines due to other rare earth elements are sometimes superimposed upon those of the element being determined; as for instance the line Ce 4012·4 A. which is coincident with Nd 4012·25 A. and which becomes visible at a concentration of about 0·25 per cent. of cerium. In this case the only satisfactory alternative lines for neodymium are 4061·1 A., which is weaker and somewhat masked by background, and 4303·6 A., which is available only at concentrations of neodymium above 0·5 per cent.

Possibly the most serious hindrance has been the variable quality of the graphite. Two different effects have been encountered that have in different ways influenced the results obtained. In the first place impurities present in the graphite have seriously interfered with the rare earth and iron comparison lines which would otherwise have been selected. The graphite originally used was of American origin and contained, among other less important impurities, titanium and vanadium in varying proportions; it was frequently possible, however, to select rods that were free from these impurities and when this was done the results obtained were superior to any obtained with subsequent supplies of graphite. Latterly the supply of graphite was obtained from sources in this country and it was immediately apparent that, although free from vanadium, it was never free from titanium; in fact it contained the latter element in almost constant amount throughout all batches of rods. This has interfered with the determination of erbium by means of the line Er 3372·75 A. and more seriously with the determination of other elements by use of Fe 3370·8 A. for comparison. Although the line Fe 3369·5 A. has proved useful it is generally less satisfactory than Fe 3370·8 A. owing to its relatively weaker density.

A small amount of high-purity graphite has been obtained but, owing to its high cost, the amount of work that could be performed with it has been limited. By its use, however, it has been shown that the absence of titanium makes it possible to use with advantage the lines mentioned above.

A further disadvantage of the later supplies of graphite is that the character of the bands in the graphite arc has shown a change which gives an increased background effect. The bands are less pronounced in spectra produced in the intermittent arc with graphite electrodes than in similar spectra produced by D.C. arc excitation—markedly so when the earlier supplies of graphite were used. It appears that the newer type of graphite contributes more to the exposure relative to the sample placed upon it than did the original graphite used. The high-purity material did, however, show background effects comparable with those of the graphite of American origin. The rather smaller diameter of the high-purity graphite was disadvantageous in other respects compared with the material used in the earlier part of the work.

The well known differences in spectral response of the rare earth oxides to arc excitation will of necessity give rise to considerable differences in the lowest limit of detectability of the various elements; *e.g.*, the limit of detection of cerium in the mixed rare earth and iron oxide concentrate is about 0·1 per cent. For this reason it has been necessary in some cases to utilise the most sensitive line, whether this is the best line for reproducibility or not, but for other elements a better reproducibility may be obtained by choosing a line that may not be the most sensitive line available; preference has been given where possible to higher precision at the expense of some slight loss in sensitivity.

Calibration curves have in general been plotted as ratio of deflection rare earth to deflection iron against percentage concentration of rare earth oxide in the oxide concentrate. In order to cover the widest possible range of composition the densities of the weakest lines are such that they may sometimes fall outside the straight line portion of the characteristic curve of the plate (density 0·1 to 1·1).

Tabulated below are the line pairs used for each element determined, together with recommended concentration limits and explanatory notes.

RESULTS AND PRECISION—

The following results were obtained in the determination of rare earths in minerals. Figures obtained in control samples, where known amounts of rare earths were added, will

TABLE II

LINE PAIRS

All wavelengths are in Ångstrom units

Element	Range, %	Rare earth lines	Iron lines	Remarks
Y	0·02–1·0	3600·7	3603·2	
		3710·3	3701·1	Stronger line than 3600·7 but somewhat masked by Fe 3709·3.
		4374·9	4352·7	Note. Fe 4376·0, Gd 4373·8.
La	0·05–1·0	4333·7	4337·0	Tb 4336·5 interferes with Fe.
		4333·7	4352·7	Note. Ce 4337·8, Sm 4334·1, 4336·1.
		3988·5	4009·7	Note. Yb 3988·0, V 4332·8.
		3995·75	4009·7	
		3337·5	3370·8	Useful above 2%; weak Fe 3337·7 may interfere.
Ce	0·25–2·0	4562·4	4603·0	
		4572·3	4603·0	Ce masked by V 4571·8.
		4012·4	4009·7	Useful in absence of Nd 4012·25.
Pr	0·1–2·0	4008·7	4009·7	Note. Er 4008·0.
		3908·4	3898·0	Somewhat masked by Fe 3907·9. Note. Sc 3907·5.
Nd	0·1–2·0	4012·25	4009·7	Masked by Ce 4012·4.
		4061·1	4009·7	Somewhat masked by background.
		4303·6	4337·0	Visible at 0·5%.
Sm	0·05–2·0	3592·6	3594·6	Note. Y 3592·9.
		4577·7	4603·0	Stronger line, though some background effect.
Eu	0·01–0·5	3972·0	3977·7	Note. Fe 3971·3, Pr 3972·2.
		4661·9	4603·0	Strong line useful for quantities less than 0·1%.
		4522·6	4476·0	Useful for quantities greater than 0·2%.
Gd	0·1–2·0	4347·3	4337·0	Use Fe 4352·7 in presence of Tb. Gd 4347·3 slightly masked by background.
		3362·2	3369·5 or 3370·8	
		3350·5	3369·5 ”	
		3358·6	3369·5 ”	
Tb	0·1–2·0	3509·2	3445·2	Slightly masked by background.
		3324·4	3369·5 or 3370·8	Weaker line but the most reliable above 0·5%.
		4338·45	4352·7	
Dy	0·02–1·0	4000·45	4009·7	Note. Pr 4000·2.
		4211·7	4233·6	More sensitive line but background rather heavy.
		3531·7	3603·2	Slightly masked by background.
Ho	0·01–0·5	3456·0	3369·5 or 3370·8	
		4053·9	4009·7	More sensitive line but background rather heavy.
Er	0·02–1·0	3499·1	3445·2	For 0·5% upwards.
		4008·0	4009·7	Recommended in absence of Pr 4008·7.
		3372·75	3369·5 or 3370·8	Note. Ti 3372·8.
Tm	0·05–1·0	3462·2	3369·5 or 3370·8	
		3362·6	3369·5 ”	
		3131·3	3369·5 ”	
Yb	0·01–0·5	3464·4	3445·2	
		3289·4	3286·8	Yb 3289·4 stronger line than 3464·4.
Lu	0·01–0·5	3359·6	3369·5 or 3370·8	
		3312·1	3369·5 ”	Note. Er 3312·4.
Sc	0·01–0·5	4023·7	4009·7	
		4020·4	4009·7	Note. Er 4020·5.
		3613·8	3603·2	
		3642·8	3603·2	
Th	1·0–10·0	4019·1	4009·7	

Note—All percentages mentioned above are calculated as rare earth oxide in the total oxide concentrate. The iron line 3370·8 is superimposed upon a titanium line and is only usable if the graphite is titanium-free.

indicate the accuracy obtainable by this method. Preliminary chemical separation in this case was carried out in far greater detail than is usually required; the preparation involved about eight precipitations, so that some loss of rare earth is to be expected.

MILLIGRAMS OF RARE EARTH OXIDE PER GRAM OF ORIGINAL MATERIAL

	Found in sample	Added to sample	Found in sample after addition
Y	nil	0·00010	0·00009
La	nil	0·00020	0·00017
Ce	nil	0·0015	0·0011
Eu	0·00009	0·00022	0·00030
Yb	traces	nil	traces

Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Lu, Sc and Th were not detected in the sample.

Determination of rare earths in fluorites has also been carried out; it is considered that the fluorescence of fluorite may be due to the presence of certain elements of this group.

As a measure of reproducibility, replicate exposures were made of a number of standard mixtures. Although the number of replicates is not large the results given will afford a fair indication of the reproducibility obtained by the method.

Tests were also carried out on both types of graphite that were used in the latter part of the work, namely, the variety that contained titanium and the high-purity graphite. The test involved about ten duplicate exposures of a given standard sample on each type of graphite. Where comparison was possible, that is, for lines not affected by titanium, the two types of graphite showed very little difference in the precision obtained, but such differences as occurred seemed to be slightly in favour of the ordinary variety. Similarly, comparisons between the two iron lines Fe 3369·5 A. and Fe 3370·8 A. for the internal standard line, which could only be made on the high-purity graphite, showed again a slight advantage in favour of the stronger iron line.

The table below shows the standard deviation of ratio deflection rare earth/deflection iron, σR, which has been translated to standard deviation on the amount of rare earth present, by comparison with a standard calibration curve.

TABLE III

Element	No. of exposures	% of element	σ R	Standard deviation %
Y	12	0·12	0·09	± 10
La	12	0·25	0·08	± 8
Ce	12	0·7	0·035	± 8
Pr	16	0·5	0·085	± 15
Nd	16	0·5	0·06	± 10
Sm	16	0·5	0·055	± 10
Eu	12	0·12	0·05	± 10
Gd	14	0·66	0·055	± 10
Th	18	0·5	0·04	± 6
Dy	14	0·33	0·095	± 10
Ho	18	0·30	0·065	± 7
Er	20	0·2	0·025	± 6
Tm	20	0·3	0·05	± 9
Yb	16	0·25	0·045	± 8
Lu	20	0·2	0·035	± 7
Sc	13	0·165	0·058	± 8

CONCLUSIONS—

The technique described, by which the sample is fused *in situ* on a graphite electrode, has provided a simple and efficient method of ensuring that the material is firmly held on both the lower and the upper electrode, thereby enabling a discontinuous source to be used. In this way the characteristic properties of the intermittent A.C. arc, namely, the high sensitivity of arc excitation together with a reproducibility comparable with that of a spark source,[2] may be utilised. Furthermore, a fusion technique permits the analysis of insoluble samples which are excluded when a solution of the material is evaporated on the electrode.[3] A method for general analytical problems is proceeding on these lines.

It may also be possible by the use of a technique such as that described by Wiggins[5] to eliminate the band spectrum of a graphite arc and thereby broaden the choice of lines available for analysis.

TABLE IV

COMPARISON OF "STANDARD" AND HIGH-PURITY GRAPHITE

Number of exposures, 10

Line pair	Standard deviation %	
	Standard graphite	High purity graphite
Lu 3359·6 A. / Fe 3369·5 A.	± 5	± 7½
Lu 3359·6 A. / 3370·8 A.	—	± 7½
3372·75 A. / Fe 3369·5 A.	—	± 7½
Er 3372·75 A. / Fe 3370·8 A.	—	± 7
Ho 3456·0 A. / Fe 3369·5 A.	± 10	± 8
Ho 3456·0 A. / Fe 3370·8 A.	—	± 7
Tm 3462·2 A. / Fe 3369·5 A.	± 8	± 9
Tm 3462·2 A. / Fe 3370·8 A.	—	± 7½

SUMMARY

A method is described for the spectrographic determination of small amounts of rare earth elements present in minerals, to include all known members of the rare earth group proper, that is, the lanthanides, and other elements usually associated with the group, namely, yttrium, scandium and thorium.

The rare earth elements are first separated from the major mineral constituents by chemical means, and ignited to oxide after addition of an iron salt to serve as an internal standard. Standards are prepared from pure rare earth oxides mixed with iron oxide.

The concentrates and standards are ground with potassium hydrogen sulphate, and fused on to cupped graphite rods, pairs of which are used as electrodes in the intermittent A.C. arc discharge.

I wish to express my thanks to Dr. P. J. Hardwick of this Department for the chemical work referred to above and to Mr. C. Park for carrying out some of the spectrographic operations and finally to the Government Chemist for permission to publish this report.

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DISCUSSION

Mr. J. **HASLAM** asked if Dr. McClelland had considered the possibility that differences in the behaviour of the iron oxide might be associated with different contents of Fe_3O_4.

Mr. B. S. **COOPER** thought that the differences might be related to size and shape of particles and suggested a closer examination of X-ray patterns and if that gave no information a comparison of samples by means of the electron microscope.

Dr. D. M. **SMITH** said that, although in recent papers he and G. M. Wiggins had stressed the advantages of the constant-current D.C. arc for work on the rare earths, they did not wish to imply that the intermittent A.C. arc was unsuitable, and he welcomed this paper as showing that under the conditions standardised by Dr. McClelland reliable quantitative data could be obtained with it.

Mr. F. J. Woodman asked if, as the rare earth oxides were presumably somewhat hygroscopic and some of them, according to the literature, were unstable on ignition, any particular precautions were taken in weighing and general handling, in the preparation of the quantitative standards used in the work described.

Dr. McClelland said, in reply to Mr. Woodman, that the rare earth oxides used in the preparation of the standard mixtures were weighed directly from the sealed tubes in which they were supplied. In the fusion technique, the temperature was raised only just sufficiently to fuse the potassium hydrogen sulphate mixture and it was considered unlikely that any loss of rare earth oxide would occur in the short time that the electrodes were maintained at that temperature.

In reply to questions on the character of the iron oxide, X-ray examination showed no differences in composition, but X-ray films would be re-examined to see if any differences in particle size had been revealed. Difference in ultimate particle size seemed to be the most likely cause of variation in character of the iron oxides and investigations on these lines would be undertaken.

Isolation and Determination of Morphine, Codeine and Heroin from Viscera and Body Fluids by Adsorption

By A. STOLMAN and C. P. STEWART

A survey of the literature shows clearly that the existing methods for the determination of morphine and its derivatives in body fluids are unsatisfactory when the amount of alkaloid is small. Even when several centigrams are present in approximately 100 g. of the material, good results can only be achieved by the most meticulous attention to the details of a long and cumbersome process.

Adsorption offers a possible alternative to the usual methods involving extraction with immiscible solvents. Mutch[1] showed that various alkaloids were adsorbed by magnesium trisilicate, though rather inefficiently under his conditions. Stewart, Chatterji and Smith[2] used adsorption on kaolin for the isolation of several alkaloids in toxicological analysis and obtained promising preliminary results. On the other hand, Daubney and Nickolls[3] discarded adsorption methods as inefficient. All three of these groups used the rather crude procedure of agitating a suspension of the adsorbent in the alkaloid-containing solution. In the toxicological analyses, moreover, it was insufficiently realised that particular adsorbents must be expected to vary in effectiveness when the extracts used as analytical material contain high concentrations of electrolytes. It therefore seemed desirable to investigate further the possibility of removing small amounts of alkaloid from tissue extracts by adsorption methods.

Experimental

Adsorbent—

We were fortunate in finding a commercially available synthetic silicate, Florisil,* which was more effective than a number of other adsorbents tested in removing morphine from pure solution. It is a hard, white granular substance, obtainable in various standard particle sizes (of which 60 to 100-mesh, standard U.S. equivalent sieves, proved most satisfactory) and, in distilled water, gives a suspension having pH 9·8. Its physical properties make Florisil particularly convenient in use, as it allows fairly rapid percolation of fluids and does not readily clog. For use it was cleaned by heating under reflux with a mixture of formic acid, ethyl alcohol and ethyl acetate in the apparatus described below.

Reagents—

The purity of the reagents was found to be an important factor.

Ethyl alcohol and methyl alcohol—Each litre of the 95 per cent. alcohol, laboratory grade, was mixed with 4 g. of silver nitrate dissolved in a minimum quantity of water and about 1 ml. of 40 per cent. sodium hydroxide solution. After the mixture had been allowed to stand for 24 hours, more sodium hydroxide was added until no further precipitation of silver occurred. The precipitated silver salt was removed by filtration and the alcohol heated under reflux for half an hour and then distilled.

Ethyl acetate—Each litre of this reagent was heated under reflux for half an hour with approximately 25 g. of phosphorus pentoxide and then distilled.

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* Obtainable from the Floridin Co. Inc., Penna, U.S.A. Manufactured in accordance with U.S. Patent No. 2,393,025.

Oxalic acid—This reagent was rendered anhydrous by heating to between 60° and 70° C. and then purified by sublimation at temperatures between 140° and 157° C. No special apparatus was required. The sublimation was carried out in a large glass-stoppered conical flask partially immersed in an oil-bath at the stated temperature. The sublimed product condensed on the cooler surfaces of the flask.

Formic acid—Analytical reagent grade of 90 per cent. formic acid was used.

Sodium carbonate solution—A saturated solution of the pure salt (AnalaR) was used.

Formaldehyde - sulphuric acid reagent—1 ml. of 40 per cent. formaldehyde solution was mixed with 99 ml. of pure sulphuric acid (sp.gr. 1·84).

Formaldehyde - ferric sulphate - sulphuric acid reagent—0·2 ml. of the formaldehyde - sulphuric acid reagent was mixed with 60 ml. of sulphuric acid (sp.gr. 1·84) and then 8 ml. of 10 per cent. (w/v) aqueous ferric sulphate solution were added with cooling.

Phenol reagent—Commercially prepared Folin - Ciocalteu reagent was used.

APPARATUS—

Adsorption columns—The columns were prepared in Pyrex glass tubes of 14-mm. diameter and 90-mm. or 120-mm. length. The Florisil was packed in the usual way (Zechmeister and Cholnoky[4]) on a base of cotton wool with the aid of a wood plunger, and was covered by a small wad of glass wool to prevent disturbance of the upper surface during manipulation.

Elution apparatus—In order to avoid the distribution of 1-mg. quantities of alkaloid Cholnoky[4]) in a large volume of solvent, elution of the adsorbed alkaloids was carried out in the apparatus shown in Fig. 1, which was made in the laboratory. A 100-ml. flask is used to contain the solvent. The lower ground glass joint can be either a B19 or B24 and the upper one a B24 or B32. Any type of condenser can be used, provided that its lower end has an extension centred over the funnel, to facilitate the flow of the condensed solvent into the adsorption tube. The stem of the inserted funnel is of sufficient length to enter the neck of the adsorptiontube. Four projections on the inner side-walls of the body of the apparatus act as supports for the adsorption tube, without hindering the return flow of the solvent into the flask. The opening at the bottom of the body for the return flow is 1 to 1·5 mm. in diameter. This eluting apparatus worked very efficiently. Very little vapour came up through the bottom and at no time was there interference with the return flow of the liquid.

The preliminary cleaning of the adsorption columns and the elution of the alkaloid were both accomplished in the eluting apparatus. The procedure was simple. The cleaning or eluting solvent was boiled at a rate so adjusted as to ensure a constant small layer of fluid above the Florisil. At times the rate of percolation through the column was slow at the start, but it invariably increased to a satisfactory rate within a short time. In order to detect the occasional sudden formation of an air lock in the top part of the column only a small layer of liquid, which served as an indicator for the rate of percolation of the solvent through the column, was permitted to collect above the Florisil. Moreover, if the adsorption tube above the adsorbent was filled to overflowing with the liquid, there was no certainty that the liquid was percolating through the column.

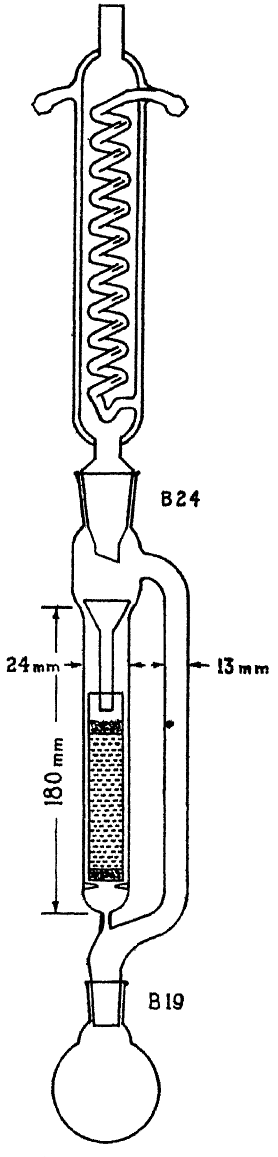

Fig. 1. Apparatus for elution with small volume of solvents

In the procedures where the Florisil was overlaid with a salt necessary for the required elution, the salt was placed on the glass wool mat and then covered with a wad of cotton wool. The use of the latter was to prevent the drops of solvent from falling on the salt and splashing it over the sides of the adsorption tube. It was not uncommon for an air-lock to form between the salt layer and the adsorbent. This, however, was easily remedied by discontinuing the heating for a minute or two, and then disconnecting the condenser and inserting a wire through the funnel into the salt layer to break the air-lock.

QUANTITATIVE METHODS USED—

No attempt was made to develop new methods of determination for the three alkaloids. With the small amounts used in this investigation it was found expedient to adopt existing colorimetric methods.

The reagents containing sulphuric acid are very liable to give "off colours" with slightly impure samples of the alkaloids, but it should be emphasised that the alkaloids adsorbed on Florisil and eluted as described invariably gave colours as true as those given by the standard solutions prepared from pure alkaloids. This is an important advantage of the adsorption procedure in the actual identification of the alkaloids.

(*a*) *Determination of morphine*—The Oberst method[5] for the colorimetric estimation of morphine was used, with substitution of the Folin - Ciocalteu phenol reagent[6] for the Folin - Denis reagent. The morphine solution, prepared from the residue after evaporation of the eluate, was transferred to a 100-ml. volumetric flask and made alkaline with 20 ml. of saturated sodium carbonate solution; 2 ml. of the phenol reagent were then added and the solution made up to volume. An intense blue colour developed rapidly, reaching maximal intensity within 2 hours and then remaining stable for many hours. All comparisons of the colour were made after 2 hours, in a photo-electric colorimeter, using a red filter (Ilford 204).

A freshly prepared heroin hydrochloride solution having the same concentration as the morphine solution gives only a faint trace of a blue colour with this reagent. A solution that has stood for several days gives a considerably deeper colour, indicating a slow hydrolysis of the heroin with liberation of the phenolic group. A pure codeine solution gives no colour with the phenol reagent.

(*b*) *Determination of heroin*—With the formaldehyde - sulphuric acid reagent, heroin gives first a red colour changing gradually to reddish-blue.

The dried residue obtained after the evaporation of the eluate was mixed with the reagent and stirred until completely dissolved. The volume was then made up in a volumetric flask to 50 ml. with more of the reagent. The colour became stable within ½ to 1 hour and readings were made after 1 hour, in the photo-electric colorimeter, using the red filter (Ilford 204). The standard follows Beer's law for low concentrations.

Morphine and codeine give colours similar to that given by heroin with this reagent, which is non-specific; although the colour shades differ, the method can only be used for determination of a single pure alkaloid.

(*c*) *Determination of codeine*—The formaldehyde - ferric sulphate - sulphuric acid reagent of Fulton[7] serves as a convenient colorimetric reagent for codeine, giving a reddish-blue colour. In the original paper 0·6 ml. of formaldehyde - sulphuric acid reagent was suggested, but we found 0·2 ml. of this reagent preferable.

The method can be standardised with any quantity of formaldehyde reagent, but the blue colour predominates with the smaller amounts of formaldehyde. The dried residue left by evaporation of the eluate was completely dissolved in some of the reagent, and the volume made up to 50 ml. with more of the reagent. The colour was read, after 1 hour, in the photo-electric colorimeter, using the red filter (Ilford 204). There was a linear relation between colorimeter readings and alkaloid concentration within the range used. Like the heroin colour reagent, this reagent is non-specific, giving colours with morphine and heroin similar to that obtained with codeine.

DEVELOPMENT OF THE METHOD

BASIC CONDITIONS OF ADSORPTION AND ELUTION—

One milligram of the alkaloid (morphine, heroin or codeine) was dissolved in 100 ml. of distilled water and the solution was run through a column of untreated Florisil. The adsorbed alkaloid was eluted by refluxing with methyl alcohol for 1 hour. Quantitative determination of the eluted alkaloid showed an apparent recovery of 105 to 120 per cent. with morphine or codeine and 94 per cent. with heroin.

Cleaning of the Florisil columns by refluxing for about 2 hours with a mixture of formic acid (1 vol.), ethyl alcohol (2 vols.) and ethyl acetate (2 vols.) in the apparatus described, and then washing with 250 ml. of distilled water, the last 50 ml. of which were used for pH determination, removed the interfering impurities and improved the adsorption of heroin so that recovery was nearly complete for all three alkaloids. The slight loss of alkaloid observed might be due to incomplete adsorption or incomplete elution or both. Obviously the elution must first be made optimal. Increasing the time of elution within reasonable limits did not solve the problem. The treatment of the columns with formic acid had decreased its "alkalinity" to the range pH 7·0 to 7·5 (*i.e.*, the washing water from the Florisil so treated had a pH lower than that from untreated Florisil and even, with long refluxing, a pH below 7·0). Any columns from which the washing water had a pH below 7·0 were discarded. It was considered that elution might be helped by restoring the alkalinity of the column, and therefore solid sodium carbonate was placed on top of the column before elution With this procedure the solvent returning to the flask from the column is strongly alkaline. Since morphine is somewhat unstable in hot alkaline solution it was considered necessary to maintain an acid reaction in the flask by addition of a suitable non-volatile acid. This was achieved by using as the eluting fluid in the flask 25 ml. of methyl alcohol containing 0·5 g. of pure oxalic acid. With this procedure the recovery of the alkaloids became complete and exact. After the elution period of 45 to 60 minutes, the alcohol solution was transferred to an evaporating dish or beaker. Distilled water in sufficient amounts was used to complete the transfer. The solution was placed on a water-bath for evaporation to a small volume. For morphine the volume was reduced only to 10 to 15 ml., as its colorimetric determination was made in aqueous solution. For codeine and heroin, the solution was evaporated on the steam-bath to the first signs of salt crystallisation, never to complete dryness. For the final stage of evaporation, *i.e.*, complete drying, the beakers were placed in a warm air current (40° to 45° C.). Overheating on the steam-bath is a source of error generally overlooked, yet it is essential to avoid it, as heroin and codeine in minute quantities can be destroyed rapidly by excessive heat.

APPLICATION TO SOLUTIONS RESEMBLING TISSUE EXTRACTS—

Our problem was to determine the alkaloids present in tissue extracts, whether prepared by the Stas - Otto process (dilute alcohol), the Dragendorff method (acidified water) or with trichloroacetic acid as suggested by Stewart *et al.*,[2] although we were not concerned, at this stage, with the efficiency of these processes in extracting the alkaloids from tissue. We had therefore to consider the effects, upon adsorption, of high concentration of salts and organic acids and of alcohol. Further, such extracts are not absolutely free from proteins and lipoids.

To simulate these conditions we used solutions containing 1 mg. of alkaloid with 1·0 to 4·0 g. of sodium chloride per 100 ml., and to these we added 25 to 150 ml. of ethyl alcohol, or 5 to 20 g. of trichloroacetic acid, or both of these reagents within the ranges mentioned.

After one of these solutions had percolated through the column, the column was washed with about 50 ml. of aqueous alcohol (4 vols. of water : 1 vol. of alcohol) and excess fluid was forced out before elution began.

When ethyl alcohol formed half or more of the total volume of the solution, adsorption was incomplete, but when it formed only one-third or less it did not appreciably interfere (Table I *a*).

With the solutions containing 5 per cent. of trichloroacetic acid, the adsorption was incomplete even when the alcohol was present in amounts which, alone, did not decrease adsorption efficiency (Table I *b*).

These solutions, of course, were strongly acid (pH about 2·0), whereas all solutions previously tried had been neutral. We therefore repeated the series, adding sodium hydroxide solution to neutralise the trichloroacetic acid (final pH about 6·5 to 7·0). Under these conditions adsorption was again complete, provided the alcohol concentration was not excessive (Table I *c*).

The object of testing solutions containing both alcohol and trichloroacetic acid was to prepare the way for a composite method of obtaining tissue extracts by adding trichloroacetic acid to a Stas - Otto or Dragendorff extract to precipitate protein, or by adding alcohol to a trichloroacetic acid extract to prevent adsorption of residual pigments.

Consistently complete adsorption of various amounts of alkaloid was achieved with trichloroacetic acid (neutralised) concentration of about 5 g. in 125 ml. of solution containing 25 ml. of ethyl alcohol. With these proportions the sodium chloride concentration could safely be raised even to 4 g. per 100 ml. It was even possible to raise the concentration of trichloroacetic acid to 16 g. per 100 ml. (Table I *d*). This shows that the conditions are remarkably elastic. In fact, the lower concentration of 1 g. of sodium chloride, 5 g. of trichloroacetic acid and 25 ml. of ethyl alcohol per 125 ml. represents the usual tissue extract, and in any case such an extract can in practice be diluted to any desired extent, since increase in volume has no other effect than to increase the percolation time.

TABLE I

ADSORPTION OF MORPHINE FROM AQUEOUS-ALCOHOL SALT SOLUTIONS

	Morphine added mg.	NaCl g.	Water ml.	C_2H_5OH ml.	CCl_3COOH g.	$CH_3COOC_2H_5$ g.	Morphine recovered %
a	1·0	—	100	100	—	—	85·0
	1·0	—	100	50	—	—	99·7
b	1·0	1·0	100	75	5·0*	—	85·0
	1·0	1·0	100	50	5·0*	—	92·0
	1·0	1·0	100	25	5·0*	—	95·0
c	1·0	—	100	—	5·0	—	100·0
	1·0	—	100	—	20·0	—	98·0
	1·0	1·0	100	150	5·0	—	80·0
	1·0	1·0	100	100	5·0	—	81·0
	1·0	1·0	100	50	5·0	—	99·5
	1·0	1·0	100	25	5·0	—	100·0
d	0·1	1·0	100	25	5·0	—	99·5
	0·2	1·0	100	25	·0	—	100·0
	0·5	1·0	100	25	·0	—	100·0
	2·0	1·0	100	25	·0	—	100·0
	5·0	1·0	100	25	·0	—	99·[illegible]
	1·0	4·0	100	25	20·0	—	99·0
	1·0	1·0	100	25	·0	5·0	100·0
	1·0	1·0	100	25	·0	10·0	100·0
	1·0	1·0	100	50	·0	20·0	85·0
	1 0	1·0	100	50	5·0	10·0	95·0

* Not neutralised.

In a few experiments it seemed that the presence of a little ethyl acetate or *iso*butyl alcohol improved adsorption. Quantitative experiments, however, showed that this was not so, but that ethyl acetate did not prevent adsorption when added in amounts up to 10 ml. per 125 ml. of the mixed alcohol - saline - trichloroacetate solution (Table I *e*).

EFFECT OF THE pH OF THE SOLUTION ON ADSORPTION—

Adsorption of morphine was complete at pH 6·5 to 7·0 but was incomplete in 5 per cent. (w/v) solution of trichloroacetic acid (pH about 2·0), and at this low pH codeine and heroin were also incompletely adsorbed. The importance of pH had also been shown by the necessity of treating the adsorbent with acid to ensure complete adsorption of heroin. Evidently more detailed examination of the effect of pH was necessary. Using the standard treatment of the column already described, we measured the recovery of each of the three alkaloids (in separate solution) from solutions of which 125 ml. contained 1·0 mg. of alkaloid, 100 ml. of 1 per cent. (w/v) sodium chloride solution, 25 ml. of ethyl alcohol and 5 g. of trichloroacetic acid, the pH being adjusted to the desired level (measured electrometrically) by titration with sodium hydroxide solution. As Table II shows, recovery of all three alkaloids was

TABLE II

ADSORPTION OF 1 MG. OF ALKALOID FROM SOLUTIONS AT DIFFERENT pH VALUES

pH of solution	Morphine %	Codeine %	Heroin %
9·0	98·0	—	—
8·0	100·0	100·0	99·3
7·0	100·0	100·0	100·0
6·5	100·0	100·0	100·0
6·0	100·0	100·0	100·0
4·0	95·9	100·0	83·8
2·0	84·0	77·0	—

substantially complete within the pH range 6·0 to 8·0. Above pH 8·0 there were indications that morphine was not quite completely adsorbed; below pH 6·0 adsorption of morphine and heroin was incomplete, but codeine was still adsorbed at pH 4·0, though not at pH 2·0.

METHOD APPLIED TO TISSUE EXTRACTS, BLOOD FILTRATES AND URINE FILTRATES

PREPARATION OF TISSUE EXTRACTS—

(*a*) For the alcoholic extracts, 100 g. of liver were macerated in a Waring Blendor with 200 ml. of 95 per cent. alcohol and enough tartaric acid to give an acid reaction. This mass was mixed with another 300 ml. of alcohol and then filtered. The filtrate was clear and deep yellow in colour. Each volume of filtrate was mixed with 4 volumes of 5 per cent. (w/v) aqueous trichloroacetic acid solution and filtered, in order to remove some of the proteins which were dissolved by the alcohol and which, if allowed to remain, were partially adsorbed by the Florisil. This filtrate was used for adsorption experiments after the adjustment of the pH and the addition of specified amounts of alkaloid.

(*b*) For the preparation of the aqueous trichloroacetic acid extract the method of Stewart, Chatterji and Smith[2] was used. Small pieces of liver (totalling 100 g.) were macerated in a Waring Blendor with 200 ml. of 10 per cent. (w/v) trichloroacetic acid solution. The filtrate obtained was clear and light yellow in colour. Each volume of filtrate was mixed with an equal volume of water in order to reduce the concentration of trichloroacetic acid to 5 per cent. For adsorption work, each 100 ml. of the 5 per cent. trichloroacetic acid solution was mixed with 25 ml. of 95 per cent. alcohol, which had been found to prevent the adsorption of the impurities by the Florisil but to cause no interference with the adsorption of the alkaloids (Table I).

PREPARATION OF BLOOD FILTRATES—

Trichloroacetic acid precipitation of the blood proteins produced a water-clear filtrate. One volume of blood was mixed with 1 volume of a 10 per cent. (w/v) aqueous solution of trichloroacetic acid and one-half volume of 95 per cent. alcohol. The coagulated proteins were removed by filtration and the filtrate was used for adsorption experiments after the adjustment of the pH and the addition of a known amount of alkaloid.

PREPARATION OF URINE FILTRATES—

Deep yellow-coloured normal human urine was used. As the urine contained a negligible amount of protein the use of trichloroacetic acid was unnecessary. The urine was mixed with an equal volume of water and half of its volume of 95 per cent. alcohol. The solution was filtered if necessary and, the pH having been adjusted, the alkaloid was added for adsorption measurements.

RECOVERY OF ADDED ALKALOIDS—

Tissue extracts, blood filtrates and urine filtrates, treated in the same way as the solutions in the "model" experiments, behaved peculiarly in that the recovery of added alkaloid was very small. These fluids had a final pH of about 6·5. Since the model solutions gave good adsorption over the range pH 6·0 to 8·0, it was obviously necessary to test the tissue extracts over the whole of this range. It was then found that the tissue, blood and urine extracts allowed complete adsorption only on the alkaline side of neutrality and indeed at a pH higher than is satisfactory for the model solutions, the optimal range being pH 8·0 to 9·0. There are several possible explanations of this discrepancy, but as yet we are unable to say which is correct.

Urine filtrates, and to a less degree tissue extracts, still contain pigment which is partially adsorbed under our conditions, and since the pigments are eluted by methyl alcohol, they interfere with the colorimetric determination of the alkaloids. They can, however, be removed by washing the column, prior to elution, with 200 ml. of a mixture containing water, ethyl alcohol and ethyl acetate in the proportions 10 : 3 : 2 by volume. This treatment completely removed the pigment from the column without disturbing the alkaloid.

One difficulty remained in the case of extracts that still contained traces of protein. Part of this protein was adsorbed and eluted with the alkaloid, appearing as a flocculum during the evaporation of the eluate. When this occurred the fluid was filtered through a small wad of lightly packed cotton wool and the filtrate, together with the washings (of 1 : 4 alcohol - water mixture) was evaporated to the required state for colorimetry.

With the method modified in these ways each of the three alkaloids gave complete recovery (Table III). Although the Folin - Ciocalteu reagent, used for the final determination of the morphine, reacts with other phenolic substances, blank controls on the extracts showed either no colour at all or only a negligible trace. We have reason to believe that the method can be applied successfully to much smaller amounts of alkaloid than 0·5 mg., the limiting factor being rather the sensitivity of the quantitative methods of determining the alkaloid in the final eluate than the manipulations involved in the adsorption and elution.

TABLE III

RECOVERY OF ALKALOIDS ADDED TO EXTRACTS OR FILTRATES

Alkaloid	Amount added mg.	Extract or filtrate of—	Volume of extract ml.	Alkaloid recovered mg.
Morphine	1·00	Tissue	250	1·00
	0·50		200	0·50
	1·00	Urine	200	0·99
	0·50		250	0·50
	0·50	Blood	225	0·50
Codeine	1·00	Tissue	200	1·00
	0·50		250	0·49
	1·00	Urine	250	1·00
	0·50		250	0·49
	0·50	Blood	225	0·49
Heroin	1·00	Tissue	200	0·98
	0·50		250	0·48
	1·00	Urine	100	0·99
	0·50		250	0·48
	0·50	Blood	200	0·50

SUMMARY

Morphine, codeine, or heroin, in amounts ranging from 0·5 to 5·0 mg., contained in 100 to 250 ml. of alcohol or trichloroacetic acid extract of tissue, blood or urine, can be quantitatively adsorbed on a synthetic magnesium silicate (Florisil) and quantitatively eluted by methyl alcohol. The conditions are discussed and a method of determination is developed.

The eluted alkaloids are shown, by the purity of their colour reactions, to be readily identifiable. The method is therefore convenient for this purpose as well as for quantitative analysis.

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The Separation and Determination of Mixtures of Morphine, Heroin, Codeine and Barbiturates by Adsorption

By A. STOLMAN and C. P. STEWART

SINCE it had proved possible, by a process of adsorption and elution, to determine accurately as little as 0·5 mg. of morphine, codeine or heroin in extracts of body fluids,[1] it seemed desirable to investigate possible extensions of the method—to the differentiation of alkaloids present together, to the separation and identification and determination of alkaloids other than the morphine group, and to the separation of non-alkaloidal toxic substances. Adsorption techniques may eventually replace the classical but less convenient procedure of fractional extraction with various solvents as a general procedure in toxicological analysis.

In this paper we describe preliminary experiments in this direction—showing the possibility of separating completely such closely related alkaloids as morphine and heroin, as well as the possibility of determining barbituric acids in presence of morphine.

EXPERIMENTAL

The adsorbent, reagents and (except as described below) the conditions were as previously described by us.[1]

SEPARATION OF MORPHINE, HEROIN AND CODEINE—

The separation of the individual alkaloids from a mixture can be accomplished only if conditions can be found for their selective adsorption or selective elution. The conditions found to be optimal for the adsorption of morphine from tissue extracts or pure solutions were satisfactory also for heroin and codeine or for a mixture of all three. The obvious line of approach therefore was to test the possibility of selective elution—either by varying the pH of the eluting solvent, or by using a variety of solvents. Some control of the pH of the solvent can be accomplished by covering the column with a layer of an acid or alkaline salt, which is slowly dissolved by the hot solvent. The control is very uncertain, however, and although there were indications that, *e.g.*, heroin was more readily eluted by ethyl alcohol at a low pH than were morphine and codeine, the results were too irregular to be useful. Selective solvents were therefore sought, and it was found that acetone would elute codeine and heroin but not morphine, whereas ethyl acetate would elute codeine but not morphine or heroin when these alkaloids were present alone. Unfortunately, when the three alkaloids were present on the column together or in pairs, these solvents gave no differential elution whatsoever.

Fractional elution having failed, we directed our attention to the possibility of selective adsorption, but here it was desirable to confine alteration of conditions to the column of adsorbent, leaving the composition of the extract unchanged and uniform for all alkaloids.

Early in our work we had attempted to re-use columns on which alkaloids had been adsorbed and then eluted with methyl alcohol; we had found that such columns would not adsorb morphine from "model" tissue extracts, but that they would still adsorb codeine and heroin. On retesting methanol-treated columns with mixtures of the alkaloids, we found that selective activity remained—no more than mere traces of morphine were adsorbed, whereas adsorption of codeine and heroin was complete. Table I shows how completely morphine could, by this means, be separated from codeine or heroin. In the experiments summarised in this table the codeine or heroin was adsorbed on a methanol-treated column (refluxed for 3 hours with methyl alcohol), eluted with methyl alcohol by the method described by us[1] and determined colorimetrically. The liquid and washings from this column were passed through a second column which had been treated in the usual way by refluxing with the formic acid - ethyl alcohol - ethyl acetate mixture; the morphine was adsorbed on this second column, from which it was eluted with methyl alcohol for colorimetric determination.

Evidently, in the small quantities tested, morphine can be successfully separated from codeine or heroin, the two alkaloids being separately determined with a high degree of accuracy. Recovery of morphine was not quite complete, which is not surprising in view of the small amount present and the extra manipulations involved in the two-column technique. There was still, however, no separation of codeine from heroin under the conditions we had imposed.

Indications have been obtained that by altering the conditions—in particular by changing the composition of the model extracts—some separation may be possible. Apart from the undesirability of such changes we have to confess that complete separation has not yet been achieved. Procedures that appeared to differentiate the two alkaloids when either was present alone, invariably failed when applied to a mixture. The heroin and codeine appeared to interfere with each other, a phenomenon similar to that previously encountered in our attempts at selective elution.

TABLE IA

SEPARATION OF MORPHINE FROM HEROIN

Mixture	Amounts mg.	Recovery: Heroin mg.	Recovery: Morphine mg.
(1) Morphine	1·0		0·96
Heroin	1·0	1·00	
(2) Morphine	1·0		0·94
Heroin	0·5	0·49	
(3) Morphine	0·5		0·49
Heroin	1·0	0·99	

TABLE IB

SEPARATION OF MORPHINE FROM CODEINE

Mixture	Amounts mg.	Recovery: Codeine mg.	Recovery: Morphine mg.
(1) Morphine	1·0		0·94
Codeine	1·0	1·00	
(2) Morphine	1·0		0·98
Codeine	0·5	0·50	
(3) Morphine	0·5		0·50
Codeine	1·0	1·00	

SEPARATION AND DETERMINATION OF BARBITURATES—

A number of experiments with Barbital (diethylbarbituric acid) and Phenobarbital (phenylethylbarbituric acid), using Florisil columns and acid or alkaline solutions similar to the model tissue extracts from which the three alkaloids had been adsorbed, showed that there was no adsorption of these barbiturates. It therefore appeared that it would be possible to separate a mixture of these alkaloids from barbiturates.

Removal of the barbiturates from the eluate of the Florisil column by adsorption on another solid was attempted. Many otherwise possible solids were unsuitable on account of their fine particle-size, which made them very inconvenient for use in an adsorption column, owing to slow percolation rate. Activated carbon with its well known adsorptive capacity for many substances, among them the barbiturates, was therefore tried for the removal of the barbiturates from the water - alcohol - trichloroacetic acid solution. Cocoa-nut shell charcoal ground to "60 to 100-mesh" particle-size was found to be the most suitable of those tried for this specific purpose.

The charcoal column was cleaned by refluxing for 2 hours with ethyl acetate in the apparatus previously described.[1] The ethyl acetate was then removed by further refluxing with ethyl alcohol and finally washing with 200 ml. of water.

The colorimetric method as described by Levvy[2] was used in this work with minor modifications. Quantitative estimations for 5 mg. of barbiturate were made by dissolving it in 2 to 3 ml. of chloroform, adding 0·2 ml. of cobaltous acetate solution and 0·6 ml. of *iso*propylamine solution. The final volume was made up to 5 ml. with chloroform. The bluish-pink colour developed immediately and was stable for more than half an hour. Comparison of the colour with that of a standard solution was made within half an hour of the development of the colour in a photo-electric colorimeter, using a blue-green filter (Ilford 302).

For the estimation of 1 mg. of barbiturate the same quantities of *iso*propylamine and cobaltous acetate reagents were used but the final volume was made up to 2 ml. with chloroform. It was not satisfactory to employ one barbiturate as a standard for the quantitative estimation of the other barbiturates, as the various barbiturates did not produce the same amount of colour per unit concentration.

Experiments with either Barbital or Phenobarbital added to model solutions containing water, salt, alcohol and trichloroacetic acid showed that the substances were adsorbed over a wide range of pH (5·5 to 8·5), but that ethyl acetate, in concentrations of only 5 ml. per 100 ml. completely inhibited the adsorption. For these experiments the adsorbed barbiturate was eluted by refluxing for 1 hour with ethyl acetate, and the eluate carefully evaporated to dryness. The crystalline residue was taken up in 10 ml. of water acidified with hydrochloric acid and the barbituric acid was extracted from this by ether, which was then evaporated to dryness, the residue being used for colorimetric determination.

In the first attempts to isolate the barbiturates from tissue extracts and urine and blood filtrates, the barbiturates were added to the filtrates from the Florisil columns on which alkaloid had been adsorbed. Precisely the same results were obtained when barbiturate, together with morphine, codeine or heroin was added to the tissue or blood extract and the alkaloid was first removed by adsorption on a Florisil column by our standard procedure. Since it had already been found that ethyl acetate interfered with the adsorption of barbiturates and since this substance was ordinarily employed in washing the Florisil columns after adsorption of alkaloids, all filtrates from the Florisil column that were to be used for the adsorption of barbiturates had to be removed before the final washing with the water - ethyl alcohol - ethyl acetate solution. The pH of the filtrate was then adjusted for, unlike the pure solution, the tissue extracts gave poor recovery of the barbiturate from the alkaline range (Table II). At pH 5·5, however, almost complete recoveries were obtained from all the solutions. Very little loss of the barbiturate was found to take place on passage through the Florisil column. Crystalline barbital was obtained from the ether residue of all the solutions tried, including the urine filtrates. No quantitative estimation of the barbiturates in the urine was attempted at this time because the urine pigments were completely adsorbed on the charcoal column and on elution a slight amount was removed with the barbiturates. This slight amount was insufficient to interfere with the crystallisation of the barbiturate but did interfere with its colorimetric estimation.

TABLE II

RECOVERY OF BARBITURATES ADDED TO TISSUE EXTRACTS, BLOOD FILTRATES AND URINE FILTRATES

Barbiturates	Amount added mg.	Solution used	pH of solution	Barbiturate found mg.
Barbital	5·0	Tissue	8·5	3·50
	5·0	Tissue	8·5	2·30
	5·0	Tissue	5·5	4·98
	1·0	Tissue	5·5	1·00
	1·0	Blood	5·5	0·99
	5·0	Blood	5·5	4·99
	5·0	Blood	8·5	4·95
Phenobarbital	1·0	Tissue	5·5	0·97
	5·0	Tissue	5·5	5·00
	5·0	Tissue*	5·5	4·98
Barbital	1·0	Tissue*	5·5	0·96
	5·0	Tissue*	5·5	4·95

* Solutions containing morphine and barbiturate percolated through a Florisil column. In the unmarked tests the barbiturate was added to the filtrate from the Florisil column.

It must be emphasised that the experiments described in this paper are of a purely preliminary character, serving only to establish the possibility of extending to mixtures and to other substances the adsorption method previously worked out for the related alkaloids, morphine, codeine and heroin. Much more detailed work is needed before the method can be accepted as of general value in toxicological analysis. The exploratory work, however, does indicate that such further work may be undertaken with reasonable hope of success, and it is for that reason that it has been reported at this stage.

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Micro-Diffusion Method for the Determination of Calcium in Blood Serum

BY M. F. MURNAGHAN

MOST of the methods for the determination of serum calcium have been based on its precipitation as oxalate, which is then determined titrimetrically (Clark and Collip, 1925[1]), colorimetrically (Sendroy, 1942[2]) or manometrically (Van Slyke and Sendroy, 1929[3]; Sendroy, 1944[4]). Titration with potassium permanganate, although the simplest and most widely used procedure, has certain disadvantages: 2 ml. of serum are generally required, the titration should be carried out at 70° to 80° C. and difficulty is experienced in obtaining a sharp end-point except in bright daylight. The manometric method possesses the advantage over the permanganate procedure that smaller amounts of serum may be used without loss of accuracy; but each estimation takes about 20 minutes, so that, if a large number are required, this method becomes time-consuming. The micro-diffusion method to be described is particularly useful where a large number of estimations have to be carried out and is as accurate as the manometric method.

In this the calcium is precipitated and isolated as oxalate, which is then dissolved in *N* sulphuric acid and oxidised by a suitable oxidant in the outer chamber of a No. 2A Conway unit[5] to carbon dioxide, which diffuses into the central chamber where it is absorbed by a barium hydroxide solution. After diffusion is complete the excess of barium hydroxide is titrated with 0·02 *N* hydrochloric acid, with phenolphthalein as indicator. A blank unit is set up at the same time, the difference between the blank and sample figures giving the amount of carbon dioxide evolved, from which the amount of oxalate present can be deduced, and from this the amount of calcium.

METHOD

REAGENTS—

Oxidant solution—Saturated ceric sulphate in *N* sulphuric acid, containing a trace of manganous sulphate.

0·05 *N Barium hydroxide solution*—25 ml. of 0·2 *N* barium hydroxide, 20 ml. of absolute alcohol, 3 ml. of 1 per cent. phenolphthalein solution in alcohol and distilled water to 100 ml.

Saturated ammonium oxalate solution—Filtered before use.

Dilute ammonia solution—2 per cent. (v/v) solution of concentrated aqueous ammonia in water.

0·02 *N Hydrochloric acid*—Prepared exactly from the *N* acid.

APPARATUS—Besides the micro-diffusion units and Conway burette, centrifuge tubes of 3-ml. capacity with pointed ends are required, and the syphoning apparatus shown in Fig. 1.

PROCEDURE—

Precipitation and isolation as oxalate—Into a 3-ml. centrifuge tube, pipette 1 ml. of distilled water and 0·5 ml. of the serum (or 1 ml. when a very low value is anticipated). Add 0·5 ml. of saturated ammonium oxalate solution, which should not touch the sides of the tube. Mix with a glass rod, and rinse the rod with distilled water until the total volume is about 3 ml. Set aside for 30 minutes and then centrifuge.

Remove the supernatant fluid with the syphoning arrangement shown in Fig. 1, until the surface of the fluid is 1 to 2 mm. above the opening in the capillary tube. The rate of syphoning should not exceed 1 ml. per minute. Rinse the sides of the tube with 2 ml. of dilute ammonia solution without disturbing the precipitate, centrifuge and syphon. Repeat the washing, centrifuging and syphoning once more and put the tube in an oven to dry at 105° C. When the tube is dry, pipette into it exactly 0·5 ml. of *N* sulphuric acid so that the acid washes the sides of the tube. Place in a hot water-bath, stir with a fine glass rod to dissolve the precipitate and allow to cool.

Determination of oxalate—Pipette exactly 0·4 ml. of the solution into the outer chamber of the unit (No. 2A). Then pipette 0·2 ml. of the 0·05 *N* barium hydroxide into the central chamber and seal the unit as quickly as possible. (Seal each unit before pipetting barium solution into the next.) Displace laterally the cork that closes the hole in the lid, rapidly pipette 0·3 ml. of the oxidant solution through the hole into the outer chamber and rapidly replace the cork to form a firm seal. Rotate the units several times, to facilitate intimate mixing, and set them aside at room temperature for 90 minutes. Then remove the lids and titrate the excess of barium hydroxide with 0·02 *N* hydrochloric acid from the Conway burette until the pink colour of the phenolphthalein disappears. Carry out blank determinations with *N* sulphuric acid.

Calculation—If 0·5 ml. of serum was taken, the volume of 0·02 *N* hydrochloric acid used, in large divisions of the burette, divided by 2 ≡ mg. of calcium per 100 ml. of the serum. (Each large division = 0·01 ml.)

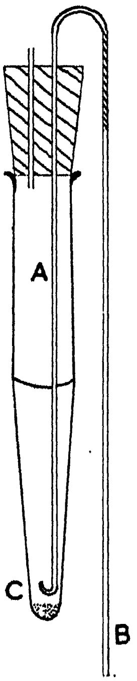

Fig. 1. Syphoning apparatus
A. Capillary with upturned end
B. Constructed outlet
C. Calcium oxalate ppt.

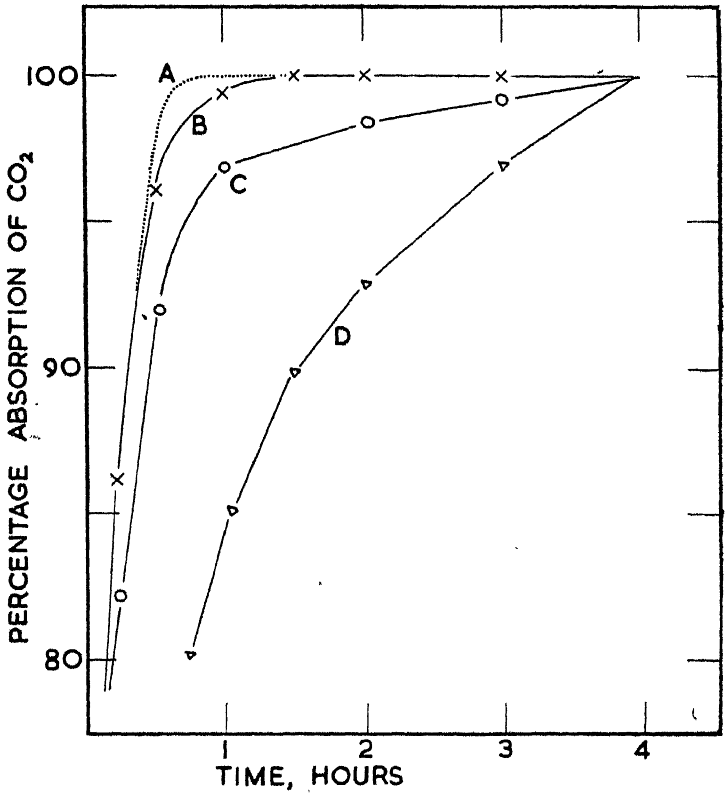

Fig. 2. Percentage absorption rates of CO_2; 0.7 ml. of fluid in outer chamber of Number 2A unit

Curve A. Acidification of bicarbonate

Curves B, C and D, 134 μg. of $(COONa)_2$ with:

B—$Ce(SO_4)_2$ (saturated) and $MnSO_4$ (trace) in *N* H_2SO_4

C—$Ce(SO_4)_2$ (saturated) in *N* or 0.2 *N* H_2SO_4

D—$Ce(SO_4)_2$ (saturated) in 5 NH_2SO_4

FACTORS INFLUENCING THE RATE OF CARBON DIOXIDE EVOLUTION—

The oxidant—Ceric sulphate has long been recognised as a suitable oxidant for oxalate. As shown in Fig. 2, Curve C, 4 hours are required for the complete evolution and absorption of the carbon dioxide from 0·4 ml. of 0·005 *N* sodium oxalate in *N* sulphuric acid with 0·3 ml. of a saturated solution of ceric sulphate in *N* sulphuric acid as oxidant. It has been found that the presence of manganese ions enhances the rate of evolution of carbon dioxide. This is shown in Fig. 2, Curve B, only 1½ hours being required for the complete evolution and absorption when a trace of manganous sulphate is added to the oxidant solution. (For comparison, Curve A shows the absorption rate of carbon dioxide liberated by acidification of bicarbonate.) A large number of trace elements, including manganese, were tried as catalysts, but they did not increase the rate of carbon dioxide production beyond that obtained with manganous sulphate. When potassium permanganate was used as oxidant, the absorption curve was similar to that with ceric sulphate alone (Curve C, Fig. 2), but permanganate was abandoned at an early stage because it involves the use of a special fixative (a mixture of paraffin wax and liquid paraffin), as permanganate reacts with petroleum jelly, the usual fixative used in micro-diffusion procedures.

Effect of acidity with ceric sulphate alone as oxidant—The absorption curve obtained when 0·2 *N* acid was used with the cerium oxidant did not differ from that with *N* acid (Curve C, Fig. 2). On the other hand, increasing the acidity to 5 *N* caused a marked decrease in the rate of carbon dioxide evolution (Curve D, Fig. 2).

FACTORS AFFECTING THE ACCURACY OF THE DETERMINATION—

In the early stages of the investigation it was noted that the coefficient of variation for the results on a given standard solution was unduly high. This was found to arise from marked variations in the time elapsing between the pipetting of the barium hydroxide solution into the central chamber and the sealing of the units. The results shown in Table I, relating to two sets of blank units (No. 2A), illustrate this point.

TABLE I

INFLUENCE OF TIME ELAPSING BETWEEN THE PIPETTING OF THE BARIUM HYDROXIDE SOLUTION INTO THE CENTRAL CHAMBER AND THE SEALING OF THE UNITS

	Rapid sealing	Delayed sealing
Number of analyses	16	15
Mean, ml. 0·02 *N* hydrochloric acid . .	0·464	0·455
Coefficient of variation	0·9%	3·0%

It can be seen that, with rapid sealing of the units after pipetting the barium hydroxide solution, (1) the mean is greater and (2) the spread is less. This is evidently due to lessened absorption of carbon dioxide from the atmosphere. Therefore sealing should be carried out immediately after pipetting the barium hydroxide solution. Assistance at this stage, to place lids and corks in position, is advisable. This principle is applicable to all estimations carried out with the Conway unit when relatively strong alkali is used as the absorbing reagent. The glass stoppers, supplied by the makers with the Conway No. 2A unit, have a diameter of only 10 mm. and perfect sealing is occasionally unobtainable. Rubber corks of about 16 to 18 mm. in diameter at the large end and smeared with vaseline always give a perfect seal.

RESULTS

(1) *Results with standard sodium oxalate solutions*—Table II illustrates the results obtained on standard oxalate solutions (0·01 *N*, 0·005 *N*, 0·0025 *N* and 0·00125 *N* disodium oxalate in *N* sulphuric acid), with 90 minutes absorption period. When only 60 minutes were allowed for absorption, the mean recoveries were 1 to 3 per cent. low.

TABLE II

RESULTS OBTAINED WITH STANDARD SODIUM OXALATE SOLUTIONS

Absorption time: 90 minutes

Disodium oxalate present, µg.	Equivalent to calcium, µg.	Disodium oxalate found, µg.	Number of analyses	Coefficient of variation, %
268·0	80	269·0	75	1·6
134·0	40	134·0	141	2·2
67·0	20	66·7	29	3·2
33·5	10	33·5	43	6·6

(2) *Results with standard calcium solutions*—Table III shows results obtained with solutions containing 10, 5, 2·5 and 1·25 mg. of calcium per 100 ml., prepared from a stock solution containing 1 mg. per ml. (5·478 g. of $CaCl_2.6H_2O$ per litre).

TABLE III

RESULTS OBTAINED WITH STANDARD CALCIUM SOLUTIONS

Absorption time: 90 minutes

Calcium present, µg.	Calcium found, µg.	Number of analyses	Coefficient of variation, %
80	81·0	30	1·4
40	40·0	41	2·2
20	20·0	22	3·8
10	9·3	18	8·6

(3) *Results on serum by micro-diffusion compared with the Clark and Collip method*—Table IV shows the results obtained on four different human sera by the two methods.

TABLE IV

SERUM CALCIUM VALUES. COMPARISON OF THE MICRO-DIFFUSION WITH THE CLARK AND COLLIP METHOD

2 ml. of serum used in the Clark and Collip method; 0·5 ml. in the micro-diffusion method

Serum	Method	Mean calcium found, mg. per 100 ml.	Number of analyses	Coefficient of variation, %
A	C. & C.	10·50	5	3·0
A	M-d.	10·49	15	2·0
B	C. & C.	12·00	3	3·5
B	M-d.	12·15	18	2·1
C	C. & C.	10·20	5	2·5
C	M-d.	10·30	8	1·8
D	C. & C.	11·00	3	3·0
D	M-d.	11·08	12	2·2

SUMMARY

1. A micro-diffusion method, using the Conway unit No. 2A, is described for estimating quantities of oxalate ranging from 30 to 270 µg., expressed as sodium oxalate (equivalent to 10 to 80 µg. of calcium). With values between 130 to 270 µg. an accuracy to within 2 per cent. is obtainable.

2. The adaptation of this procedure to the determination of 40 to 80 µg. of calcium in blood serum (about 0·5 ml.) with an accuracy to within 2 per cent. is described.

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DEPARTMENT OF BIOCHEMISTRY AND PHARMACOLOGY
UNIVERSITY COLLEGE, DUBLIN

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Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin

Part II

The Determination of Total Penicillins by the Iodimetric Method

The Sub-Committee appointed was as follows—
C. R. Bond, M.Sc.Tech., F.R.I.C., Imperial Chemical Industries Ltd. (*Chairman*).
L. J. Bellamy, B.Sc., Ph.D., Ministry of Supply.
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Introduction

In Part I of this Report,[1] we have described the determination of benzyl penicillin on a weight/weight basis by precipitation with N-ethylpiperidine. At a meeting of the Conference on March 5th, 1948, it was agreed that crystalline penicillin G offered for sale ought to have a benzyl penicillin content of not less than 90 per cent. of the total penicillins present. In order to express the benzyl penicillin content on this basis it is necessary to know the percentage of total penicillins. The Sub-Committee has therefore investigated the iodimetric method originated by Alicino[2] and subsequently modified by Mundell, Fischbach and Eble.[3] Various modifications have been employed by several members of the Sub-Committee, and comprised essentially the use of—

(1) Buffered reactants to reduce the effect of non-penicillin impurities to a minimum;
(2) Different strengths of alkali for inactivation of the penicillin;
(3) Longer or shorter periods for inactivation and iodination;
(4) Temperatures of inactivation and iodination ranging from room temperature to 30° C.;
(5) Higher concentrations of potassium iodide in the standard iodinating reagent.

These modifications are detailed for the three methods described in Table I.

Table I

Modifications in use by members of the sub-committee

	Method I	Method II	Method III[4]
Concentration of solution, mg./ml.	0·6	1·2	0·6
Volume of aliquot, ml.	5·0	10·0	5·0
Solvent	water	water	buffer
Concentration of sodium hydroxide solution	0·1 *N*	0·1 *N*	1 *N*
Volume of sodium hydroxide solution, ml.	5·0	5·0	1·0
Time of inactivation, minutes	30	60	30
Temperature of inactivation, ° C.	30	30	room temp.
Concentration of hydrochloric acid	0·11 *N*	0·11 *N*	1 *N*
Volume of hydrochloric acid, ml.	5·0	5·0	1·0
Concentration of iodine solution	0·01 *N*	0·02 *N*	0·01 *N* (in buffer)
Volume of iodine solution, ml.	15·0	25·0	10·0
Concentration of KI in iodine solution, %	0·2	2·52	0·2
Time of iodination, minutes	15	60	30
Temperature of iodination, ° C.	room temp.	30	20
Concentration of sodium thiosulphate solution	0·01 *N*	0·01 *N*	0·01 *N*
Standing time of blank, minutes	nil	nil	5
Factor (1 mg. of sodium benzyl penicillin ml. of 0·01 *N* iodine)	2·52	2·796	2·31

It will be observed that Method I is very similar to the method of Mundell, Fischbach and Eble, the two main differences being the quantity of alkali added, an amount equivalent to that used in the original method of Alicino being retained, and the introduction of a longer period and a higher temperature for inactivation.

EXPERIMENTAL

Preliminary work to determine the effects of modifying time and temperature in Method I gave the results shown in Table II.

TABLE II

EFFECTS OF TIME AND TEMPERATURE IN METHOD I

Inactivation		Iodination				
Time min.	Temp. ° C.	Time min.	Temp. ° C.	Iodine ml.	Penicillin %	Remarks
30	20	15	20	10	94·7; 95·1	
30	20	15	20	15	95·2; 95·3	
30	30	15	20	15	97·4; 97·7	*Cf.* Method I
30	30	15	30	15	97·3	
30	30	30	30	15	98·0; 98·1	
30	30	45	30	15	99·1	
30	30	60	30	15	99·4; 99·7	
30	30	75	30	15	99·5; 99·7	
30	30	90	30	15	99·0; 99·2	
60	30	30	30	15	99·5; 99·8	
60	30	60	30	15	100·9; 101·3	*Cf.* Method II

It was thus confirmed that the conditions must be carefully standardised, if consistent results are to be obtained.

The Sub-Committee decided to make a series of tests on a standard sample of crystalline sodium benzyl penicillin (the A.S.C. Standard—*vide* Report, Part I[1]) and on a crude sodium salt (B.1005), using the methods described in Table I, with a view to selecting the procedure that gave the most reproducible and uniform results.

The composition of B.1005 was as follows—

Potency u./mg. (*S. aureus*)	% Penicillin (bio-assay)	Penicillins (*B. subtilis* units) % of total penicillins (micro-chromatogram)					
		X	U_1	G	F	D	K
1120 (3)	67	trace	trace	95	2	2	1

figures in parenthesis here and in the following tables denote the number of determinations.

The results obtained are given in Tables III and IV.

TABLE III

RESULTS OBTAINED BY THE THREE METHODS ON THE A.S.C. STANDARD

Member		Method I %	Method II %	Method III %
A	Range	98·0–98·1 (2)	98·1–98·2 (2)	100·7–100·9 (3)
	Mean	98·1	98·2	100·8
B	Range	97·4–98·4 (3)	96·9–99·9 (3)	98·7–101·0 (3)
	Mean	98·0	98·4	100·1
C	Range	99·1–100·0 (6)	95·8–99·6 (8)	99·6–99·7 (4)
	Mean	99·3	96·9	99·6
D	Range	99·1–99·5 (2)	97·3–97·7 (4)	101·6–101·7 (2)
	Mean	99·3	97·5	101·7
E	Range	96·7–99·2 (15)	95·1–97·2 (12)	100·7–103·0 (10)
	Mean	97·6	96·1	101·9
Difference between extreme means	..	1·7	2·3	2·3

TABLE IV

RESULTS OBTAINED BY THE THREE METHODS ON SAMPLE B.1005

Member		Method I	Method II	Method III
		%	%	%
A	Range	64·5–65·8 (4)	65·8–66·9 (4)	66·7–69·2 (6)
	Mean	65·3	66·4	67·6
B	Range	64·8–65·8 (5)	65·4–69·5 (5)	68·7–69·2 (4)
	Mean	65·3	66·7	68·9
D	Range	67·7 (2)	69·0–69·4 (2)	72·0–72·4 (2)
	Mean	67·7	69·2	72·2
E	Range	66·1–67·1 (6)	62·9–64·5 (4)	66·3–68·4 (6)
	Mean	66·8	63·7	67·5
Difference between extreme means	..	2·4	5·5	4·7

As Method I gave rather more consistent results than the others, the use of the buffer in Method III and the addition of more potassium iodide in Method II indicating no advantages in this respect, it was adopted as the basis for further work. In Method I, however, the quantities of sample and reagents used were rather small, and it was considered that these could be increased with advantage. Preliminary experiments had shown that the degree of inactivation and iodination were affected by temperature changes; as "room temperature" was insufficiently precise, it was agreed to use 30° C., this being generally the lowest temperature that could be easily controlled.

Incorporation of these modifications provided the "Tentative Method" of the Sub-Committee, which differed from Method I in using 10-ml. aliquots of a solution of the sample containing 1·2 mg./ml. and 30 ml. of 0·02 *N* iodine containing exactly 0·4 per cent. of potassium iodide and in employing 30° C. for both inactivation and iodination.

The Sub-Committee examined by this method four samples of penicillin, ranging in purity from crystalline benzyl penicillin (the A.S.C. Standard) to a very crude sample containing about 26 per cent. of penicillin, with a view to obtaining information on the reproducibility and general level of the results with penicillins of various degrees of purity.

The composition of the two samples not already described was found to be as follows—

Sample	Potency u./mg. (*S. aureus*)	% Penicillin (bio-assay)	Penicillins (*B. subtilis* units) % of total penicillins (micro-chromatogram)						
			X	U_1	G	F	D	U_4	K
48/0058	1444 (5)	86	trace	trace	72	10	8	trace	9
M.O.S.1	438 (3)	26	—	trace	96	4	trace	—	trace

The results obtained by the different members are given in Table V.

TABLE V

RESULTS OBTAINED BY DIFFERENT MEMBERS USING THE "TENTATIVE METHOD"

Member		A.S.C.	48/0058	B.1005	M.O.S.1
		%	%	%	%
A	Range	99·5–100·6 (5)	82·0–82·4 (2)	65·3–66·8 (6)	27·3–27·4 (2)
	Mean	99·9	82·2	66·1	27·4
B	Range	102·8–103·7 (4)	84·8–85·3 (2)	—	26·4 (1)
	Mean	103·1	85·0	—	26·4
C	Range	98·0–102·0 (6)	77·5–85·0 (4)	60·7–65·9 (12)	26·2–27·1 (3)
	Mean	100·5	81·9	64·7	26·6
D	Range	101·3–102·3 (4)	85·6–87·2 (4)	65·9–68·7 (4)	28·1–28·8 (4)
	Mean	101·8	86·4	67·3	28·5
E	Range	97·3–98·8 (4)	81·0–82·0 (4)	63·9–66·2 (4)	25·2–26·8 (4)
	Mean	98·1	81·5	65·3	26·2
	Grand Mean	100·7	83·4	65·8	27·0
Difference between extreme means	..	5·0	4·9	2·6	2·3

The conclusions reached from consideration of the above results were—

(1) Member D obtained consistently higher results than the others with all samples and member B sometimes obtained higher results. Thorough examination of all reagents and working conditions, followed by exchange of reagents by members A and D, failed to account for this.

(2) Non-penicillin impurities did not have as great an effect as might have been expected.

In an effort to reduce the variation in results, further experiments were made in which the concentration of alkali present and the temperature of inactivation and iodination were varied.

The results obtained on the A.S.C. Standard, using Method I except for the modifications stated, are given in Table VI.

Table VI

Effect of alkali concentration and temperatures of inactivation and iodination on iodine absorbed

Member	Temp. of inactivation ° C.	Temp. of iodination ° C.	Alkali added 0·1 *N* 5 ml. %	0·1 *N* 10 ml. %	*N* 0·5 ml. %	*N* 1 ml. %	*N* 2 ml. %	*N* 5 ml. %
D	30	room temp.	98·0					100·3
	24	room temp.	98·0					100·3
	5	5	93·6					98·4
E	30	30	96·4	96·4		95·3	98·0	
	30	0	93·3	94·1		95·3		
	0	30	70·4	85·2		94·5		
	0	0		77·0	85·2	95·5		

These results show that variation in the temperature of inactivation has a considerable effect on the amount of iodine absorbed when inactivation is carried out with 0·1 *N* sodium hydroxide. This effect is, however, considerably reduced when *N* sodium hydroxide is used for inactivation.

Further experiments were made with the tentative method to find out whether the use of *N* sodium hydroxide in place of 0·1 *N* sodium hydroxide would so reduce the effects of small variations in temperature as to permit of the test being carried out at room temperature, with its normal fluctuations, without thereby introducing any appreciable error in results: at the same time the adequacy of the proposed periods of inactivation and iodination, using *N* sodium hydroxide, were examined. The results obtained are shown in Table VII. In each separate experiment the only variable was the one under investigation.

Table VII

Effect of variations in temperature and time of inactivation and iodination

Time of inactivation, minutes		20	30	40
Per cent. found		102·8	102·8*	102·6
Time of iodination, minutes		10	20	30
Per cent. found		102·9	102·6	102·8
Temperature of inactivation, ° C.		14	23	30
Per cent. found		102·0	101·6	101·9*
Temperature of iodination, ° C.	0	14	23	30
Per cent. found	98·2	98·6	103·8	104·1*
Temperature of inactivation and iodination, ° C.		14	23	30
Per cent. found		101·2	103·9	104·4*

* Indicates conditions of final method.

It should be noted that in Table VII the results marked with an asterisk were obtainep under the conditions of the final method. These show appreciable variation from one to another and are indicative of the variation in results to be expected with this method.

These experiments confirmed that the effects produced by variation in temperature were too large to be ignored, particularly at the iodination stage Extension of the periods of inactivation and iodination beyond those proposed had little effect on the amount of iodine absorbed. It was concluded therefore that the tentative method should be modified in the light of these data by the use of 5 ml. of *N* sodium hydroxide in place of 5 ml. of 0·1 *N* sodium hydroxide. It was obvious, however, that with the modified conditions of assay a different conversion factor was necessary. In order to determine this factor, each member made a number of determinations on the A.S.C. Standard, which contained (*vide* Report, Part I) 98·0 per cent. of penicillins, including approximately 97·5 per cent. of benzyl penicillin.

Using a conversion factor of 0·3822 mg. of sodium benzyl penicillin per 1 ml. of 0·01 *N* iodine, which preliminary work had shown to approximate to the correct value, the results given in Table VIII were obtained.

TABLE VIII

RESULTS OF DETERMINATIONS ON THE A.S.C. STANDARD BY THE FINAL METHOD

(Replicate weighings with duplicate assays)

	Sodium benzyl penicillin found, %				
Member	Weighing 1	Weighing 2	Weighing 3	Weighing 4	Mean
A	97·7 97·9	96·9 97·2	96·5 97·5	97·1 98·1	97·36
B	98·7 99·7	98·9 99·5			99·20
C	98·0 99·0	99·0 99·0	97·5 97·0		98·25
D	99·7 99·7	98·2 98·5	98·4 98·4		98·82
E	96·4 97·1	97·3 98·8	96·8 95·5	97·5 97·5	97·11

Statistical analysis of the data in Table VIII shows that there is a significant difference between laboratories and also between duplicates. Based on the mean of duplicates and on the assumption that the variation within each laboratory is the same, the variance due to error within laboratories is 0·49; allowing for this, the variance between laboratories is 0·62. The variance of each laboratory mean and the weight to be attached to each mean are given in Table IX.

TABLE IX

WEIGHT ATTACHED TO EACH LABORATORY MEAN

Laboratory	Mean, %	Variance V	Weight 1/V
A	97·36	0·74	1·35
B	99·20	0·86	1·16
C	98·25	0·78	1·28
D	98·82	0·78	1·28
E	97·11	0·74	1·35

The weighted mean is 98·11, with a standard deviation of 0·39. Approximately four degrees of freedom may be associated with this estimate of error, which gives 95 per cent. fiducial limits of 97·03 to 99·19. This is due to differences between laboratories which in practice it has not been found possible to eliminate.

Taking these data into consideration and regarding the A.S.C. Standard as 98·0 per cent. sodium benzyl penicillin, the best estimate of the conversion factor is

1 ml. of 0·01 *N* iodine $\equiv$ 0·382 mg. of sodium benzyl penicillin.

The error due to assuming the iodine absorption of pent-2-enyl penicillin to be the same as that of benzyl penicillin and to disregarding the difference in molecular weights between these two penicillins is negligible compared with the error of the method, in view of the very small amount (0·5 per cent.) of pent-2-enyl penicillin present in the A.S.C. Standard penicillin.

The method recommended is therefore as follows.

METHOD

REAGENTS—

0·1 *N* Sodium thiosulphate solution.

0·01 *N* Sodium thiosulphate solution prepared by dilution of the above daily.

0·1 *N* Iodine in 2 per cent. potassium iodide solution.

0·02 *N* Iodine solution prepared by dilution of the above daily.

1 per cent. Starch solution prepared by pouring a paste of 2 g. of soluble starch and 5 ml. of distilled water into a boiling solution of 40 g. of sodium chloride in 200 ml. of distilled water.

N Sodium hydroxide.

1·1 *N* Hydrochloric acid.

PROCEDURE—

Weigh accurately about 60 mg. of the sample, dissolve in distilled water and dilute to a volume of 50 ml. Transfer a 10-ml. aliquot to a stoppered flask, add 5 ml. of *N* sodium hydroxide and allow to stand for 30 minutes in a water-bath at 30° C. Acidify with 5 ml. of 1·1 *N* hydrochloric acid, add 30 ml. of 0·02 *N* iodine, close the flask with a wet stopper and place it in a water-bath at 30° C. for 15 minutes. Titrate the excess of iodine with 0·01 *N* sodium thiosulphate, adding 1 ml. of starch solution near the end-point.

Complete a blank determination by transferring a 10-ml. aliquot of the penicillin solution to a stoppered flask. Add 30 ml. of 0·02 *N* iodine and titrate immediately with 0·01 *N* sodium thiosulphate.

The difference between the two titrations represents the amount of iodine that has reacted with the penicillin. Each titration should be made in duplicate.

Each millilitre of 0·01 *N* iodine is equivalent to 0·382 mg. of sodium benzyl penicillin.

The Sub-Committee is indebted to all those who have assisted in the experimental work, and to Dr. O. L. Davies for his statistical analysis of the data in Table VIII.

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The Estimation of Trichloroethylene in Air

BY F. H. BRAIN

INTRODUCTION

IN the course of a recent investigation of the use of trichloroethylene in midwifery, the results of which are shortly to be published by Helliwell and Hutton, the need arose for a simple and rapid, but reasonably accurate, method for determining small concentrations of this drug in air. The methods recorded in the literature for the estimation of halogen-containing hydrocarbons in air fall into four classes.

1. Lamson and Robbins[1] and Robbins[2] utilised the effect of carbon tetrachloride vapour on the rate of loss of heat from a hot wire and were able to detect one part in 10,000 to 20,000 parts of air. Though the method is elegant it calls for somewhat specialised and expensive apparatus.

2. Dudley[3] decomposed carbon tetrachloride and trichloroethylene in a known volume of air by passing through a tube at 800° C. and Schayer and Ackermann[4] these and other chloro-hydrocarbons by combusting in a gas flame, the resulting chlorine and hydrogen chloride being absorbed in an alkaline solution of a reducing agent. The chloride ion was estimated gravimetrically, turbidimetrically or by Volhard's method. Dudley determined in this way carbon tetrachloride and trichloroethylene in concentrations down to 0·15 mg. per litre with high accuracy but required air samples of from 12 to 65 litres, the passage of which through the furnace took up to 30 minutes, whilst Schayer and Ackermann claim recoveries of 91 to 95 per cent. at concentrations of 100 to 400 p.p.m., using only 275-ml. air samples and 95 to 98 per cent. using 500-ml. samples. Tebbens[5] and Olsen, Smyth, Ferguson and Scheflau[6] have described apparatus similar to that of Dudley.

3. Wells[7] adsorbed the chlorohydrocarbon from a known volume of air on 40 to 50 g. of charcoal, the chloride content of a sample of which was determined by silver nitrate after hydrolysis with alcoholic potash or in a Parr bomb. The method was time-consuming and considerable difficulty was experienced

in obtaining uniform samples of charcoal for analysis. The quantities of carbon tetrachloride recovered ranged from 4 to 700 mg.

4. The remaining methods relied on the removal of the halogen-containing vapour from a measured quantity of air by passing it through a known volume of a solvent, the concentration in which was then determined in some convenient manner. Barrett[8] estimated trichloroethylene absorbed in alcohol from 1 to 3 litres of air, making use of the colour reaction discovered by Fujiwara.[9] The colour intensity was compared visually with those produced simultaneously from standard solutions of trichloroethylene in alcohol. The concentrations so found were always high, and to correct for this they had to be multiplied by the empirical factor 0·68 to bring them within the limits of error of ±11 per cent. It was claimed that as little as 20 p.p.m. could be detected though the error at this low concentration was extremely high. Webb, Kay and Nichols,[10] however, absorbing carbon tetrachloride by passing through a sintered glass plate in acetone, record a recovery of 144 ± 20 per cent. Rogers and Kay[11] improved on this, using fritted glass bubblers, designed by Rogers[12] for absorption and obtained recoveries of 103 ± 9, 100 ± 10 and 96 ± 7 per cent. of carbon tetrachloride at concentrations of 50, 112 and 209 p.p.m. respectively, with acetone as solvent. Recovery varied somewhat with the rate of air flow. These authors stated that, whilst 1-litre samples sufficed, they used, in fact, as much as 5 litres for concentrations of the order of 100 p.p.m. Wells[7] was unable to obtain satisfactory results in this way, absorbing carbon tetrachloride in alcohol, hydrolysing with alkali and estimating as chloride. That there should be some discrepancy in the results obtained by these authors is not surprising considering the volatility of the solvents employed and, in some cases, the volume of air sample passed through them. This difficulty was partially overcome by Powell[14] who employed toluene as solvent and designed an apparatus that had a sufficiently low air flow resistance to permit of its use for estimating trichloroethylene in expired air. This employed three absorption bottles of special design in parallel, for which the efficiency was estimated to be 92 per cent. for air streams containing up to 3·5 mg. per litre at a total rate of 1 litre per minute. The volume of toluene employed, however, amounted to 90 ml. in all and it was necessary to pass, according to the concentration, between 15 and 40 litres of air, which was subsequently collected in a Haldane bag for volume measurement.

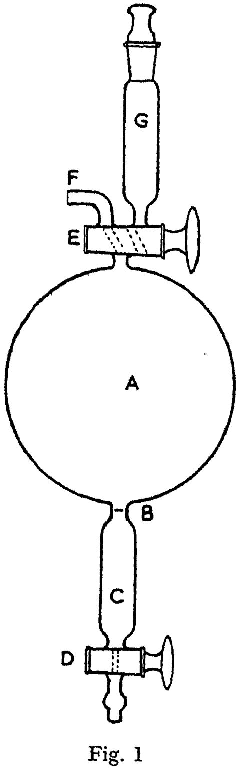

Fig. 1

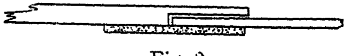

Fig. 2

For the present purpose a different method has been devised and is now described. The trichloroethylene is absorbed in anisole from an air sample of limited volume and estimated colorimetrically by means of a modified form of the Fujiwara reaction. The method presents no difficulties due to air flow and is very suitable for the estimation of trichloroethylene in expired air.

METHOD

APPARATUS—

A glass sampling and absorption apparatus, represented in Fig. 1, was constructed. In this a calibrated bulb, A, of 200 to 250-ml. capacity, is connected by a constricted section, B having a graduation mark, to a smaller chamber, C, of about 10-ml. capacity. C terminates in a tap, D, and A in a two-way tap, E, connecting it either to the air-trichloroethylene mixture via F, or to the third stoppered chamber, G, of capacity about 10 ml. The bore of E should be at least 3 mm.

Operation—After cleaning and washing the apparatus, but before drying, lubricate the taps with a paste of fine graphite and glycerine and then wash out the apparatus with water to remove surplus lubricant. Rotate each tap a number of times during the washing until the water stream, as judged by the colour imparted to it, removes only an insignificant further amount of lubricant. Then wash out the apparatus liberally with acetone, allow to drain and thoroughly dry by sucking air through it, rotating the taps until all trace of acetone has been completely removed. The taps now have dry channels from which the lubricant has been washed away, thus minimising absorption of trichloroethylene by the lubricant. After mounting the apparatus on a stand, attach a mercury reservoir to the lower extremity by rubber tubing and connect F to the source of the trichloroethylene - air mixture by a short capillary tube as in Fig. 2, to reduce contact with the rubber tubing to a minimum. A little fine graphite on the rubber tube facilitates detachment later. Fill A with mercury, expelling the air via G, and sweep out F and the connecting capillary by partially filling A via F–E and discharging via E–G. Then stopper G, fill A with air sample to the graduation mark of B and close D and E. Detach F and, after G has been blown out with clean air, introduce a known volume of anisole and stopper G. The volume of anisole used can be varied according to requirements, but 6 ml. has usually been found suitable. Then run out the remaining mercury in C, the slight reduction in pressure preventing seepage of solvent round the taps, detach the mercury reservoir and connect A and G. Distribute the anisole between A, C and G and shake the apparatus for 15 minutes. Ensure uniformity of the anisole solution by passing it to and fro once or twice between G, A and C. Finally invert the apparatus and transfer the solution and allow it to drain as completely as possible into G. Close E and sample the anisole as below.

ESTIMATION OF TRICHLOROETHYLENE IN THE ANISOLE SOLUTION—

The method employed for estimating the trichloroethylene in the anisole extract, depending on the measurement of a cherry-red colour formed on heating an aliquot portion with pyridine and 40 per cent. sodium hydroxide solution, has been fully described by Brain and Helliwell.[14] The method is summarised below.

REAGENTS—

Pyridine—"Pure anhydrous" or AnalaR pyridine, dried over potassium hydroxide sticks and redistilled. Lower grade pyridine should not be used.

Anisole—washed twice with dilute sodium hydroxide solution and three times with distilled water, dried over anhydrous calcium chloride and redistilled.

Sodium hydroxide solution—10 *M* in water, standardised.

Tetraethylammonium hydroxide reagent—prepared by adding to 10 *M* sodium hydroxide sufficient of the quaternary hydroxide to make it 0·025 *M* with respect to that base.

PROCEDURE—

Measure with a pipette a suitable aliquot of the anisole solution and sufficient pure anisole to make the combined volumes up to exactly 5 ml., and add both to 5 ml. of dry colourless redistilled pyridine and 3 ml. of 10 *M* sodium hydroxide in a 6-inch by ¾-inch Pyrex test tube; immediately place the test tube in a boiling water-bath and stir the mixture mechanically for 10 minutes at such a rate as to ensure thorough mixing of the two layers. This and the subsequent operations must be carried out in subdued daylight or artificial light, as bright sunlight causes rapid fading of the colour. Then remove the water-bath, add exactly 1 ml. of the tetraethylammonium hydroxide reagent to the mixture, continue stirring for exactly 1 minute, remove the tube and cool thoroughly. The two layers separate rapidly. Remove the upper (pyridine) layer by means of a pipette that has been swept out with carbon dioxide-free air (acid gases discharge the colour) and deliver it into a prepared 1-cm. absorptiometer cell by admitting carbon dioxide-free air to the pipette. The cell should have been prepared 10 minutes before use by placing in it 0·5 ml. of 10 *M* sodium hydroxide and covering with a vaselined slide, which is displaced to admit the coloured solution and then replaced. Measure the colour intensity immediately with a Spekker photo-electric absorptiometer, using a water cell in comparison and Ilford Spectrum Blue-green filters No. 603. The colour is stable in subdued light for about 20 minutes, allowing ample time for a series of readings to be taken. The first reading of a series is sometimes a trifle low.

Calibration of the absorptiometer—Standard solutions of trichloroethylene were prepared as follows. The mean weight, *W*, of anisole delivered by the 5-ml. pipette reserved for this solvent was determined. A sealed thin-glass ampoule containing approximately 80 mg. of trichloroethylene, accurately weighed, was broken under a known weight of the anisole in a stoppered bottle and the solution thoroughly mixed. This primary standard was then broken down in steps, by weight, until a solution was obtained containing exactly 10 mg. of trichloroethylene per *W* g. of solution. From this, sub-standards were prepared by dilution by volume, using always the same pipette for both trichloroethylene solution and pure anisole. Five-ml. portions of each sub-standard were submitted in duplicate to the colour development process described above and a calibration equation derived from the mean Spekker readings so obtained for each concentration.

In the results recorded in Table I the relationship between the Spekker drum readings, S, and the weight of trichloroethylene, T mg., in the volume of anisole solution taken were related by the expression $T = 0{\cdot}1434\ S$. It was convenient if the volume of anisole used for extraction and the size of the aliquot taken were such as to give a Spekker reading of the order of 0·5 to 0·8. Each estimation (excluding the time taken in preparing the trichloroethylene - air mixtures for the experiments recorded in Table I) required approximately 1 hour and the apparatus, after cleaning the taps, washing with acetone and flushing well with warm water, was ready for use again.

RESULTS—

In testing the apparatus, standard trichloroethylene concentrations were put up as follows. Standard solutions of trichloroethylene in dry redistilled benzene were prepared by weight and the mean weight of each solution delivered by a 1-ml. pipette was determined. One ml. of each solution in turn was delivered into a dry, calibrated, approximately 10-litre stoppered bottle, which was immediately stoppered and inverted into a bowl of mercury and left for 2 hours. This time was found to be sufficient for uniform distribution of the vapour. In calculating the actual trichloroethylene concentration it was assumed that the small quantity of benzene present augmented the volume of mixture as if it were behaving as a perfect gas at N.T.P. The sampling apparatus was prepared, and F (see Fig. 1) was attached to a bent capillary tube long enough to reach to the bottom of the 10-litre bottle and provided with a cardboard collar to cover the mouth of the bottle when, after unstoppering, the capillary tube was thrust into it. The sample was taken, extracted and estimated as described above.

TABLE I

SUMMARY OF RESULTS

Volume of bottle, 11·25 litres. Correction for volume of benzene vapour, 0·24 litre.
Volume of anisole used for extraction, 6 ml. Volume of air sample taken, 223 ml.

Concentration of trichloroethylene taken		Volume of anisole extract used	Number of estimations	Concentration found		Mean recovery for each concn. range
mg./litre	p.p.m.	ml.		mg./litre	%	
5·31	904	0·5	1	5·12	96·5	
5·76	981	,,	5	5·54–5·79	96·1–100·3	98·1%
1·83		2·0	1	1·74	95·0	
1·76	300	,,	1	1·74	98·6	
1·70		,,	1	1·68	98·5	
1·59		,,	1	1·56	98·0	
1·15	196	,,	1	1·14	98·9	97·8%
0·525		5·0	3	0·512–0·532	97·5–101·1	
0·500	85	,,	7	0·502–0·527	100·3–105·1	101·3%
0·351	60	5·0	2	0·366–0·368	104·2–104·8	104·6%
0·175	30	5·0	4	0·175–0·181	100·0–103·5	
0·168		,,	2	0·170–0·173	100·9–102·8	101·4%

Over-all mean recovery 100·3%
Average deviation from the mean 2·3%

TABLE II

Trichloroethylene, vols. % P	Concentration, C, in mg./100 ml. of blood			
	Sample A		Sample B	
	Found, C_A	Calculated*	Found, C_B	Calculated†
0·53	1·84	1·85	2·15	2·21
0·70	2·30	2·45	2·77	2·91
1·49	5·29	5·21	(6·76)§	6·20

* $C_A = 3{\cdot}50\ P$ †$C_B = 4{\cdot}16\ P$

§ This figure is too high, as the average value of the ratio C_B/C_A for the figures given, together with others, not quoted, for which P was not determined, was 1·19 (*i.e.*, 4·16/3·5), and C_B for a value of $P = 1{\cdot}49$ should therefore be of the order 6·2 mg./100 ml.

An account of the use of the method by Helliwell and Hutton in connection with work on trichloroethylene as an anaesthetic for midwifery purposes will appear elsewhere in due course. I am indebted to them for permission to include the following figures amongst the results of this preliminary investigation. The table below records the concentration, C, of trichloroethylene in mg. per 100 ml., determined by the method of Brain and Helliwell,[14] in two samples of blood exposed to three different partial pressures of the drug, P, expressed in volumes per cent., which were estimated by the method described above. C should, of course, be proportional to P and this is verified for sample A and to a lesser extent for sample B, bearing in mind the normal variation of estimations on physiological material. Values calculated on the assumption of such a proportionality are included for comparison.

SUMMARY

A method is described for estimating the concentration of trichloroethylene in air samples. It has been shown to give recoveries of 95 to 105 per cent. with concentrations from 5·76 down to 0·17 mg. per litre (981 to 29 p.p.m.) using only 223 ml. of the air sample. Of the twenty-nine estimations recorded, nineteen gave results within the limits 100 ± 2 per cent. and the mean of all was 100·3 per cent.

The method would appear to be applicable to the estimation of other substances.

The cost of materials used in this work was covered by the Association of Anaesthetists of Great Britain and Ireland.

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Addendum to paper by Klatzkin, Norris and Wokes in the August issue, 1949, p. 454. Please add the following acknowledgement in the final paragraph—

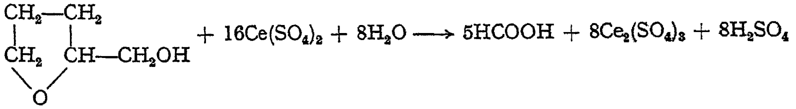
"Our thanks are due to our colleague Miss Nora Baxter for providing spectroscopic data."

Notes

NOTE ON THE DETERMINATION OF SMALL AMOUNTS OF TETRAHYDROFURFURYL ALCOHOL

RECENTLY we were called upon to determine small amounts of tetrahydrofurfuryl alcohol in aqueous solution. It was shown that this alcohol could be determined by oxidation with ceric sulphate in sulphuric acid solution at boiling temperature.

The oxidation appears to proceed as follows:—



$$C_4H_7O{-}CH_2OH + 16Ce(SO_4)_2 + 8H_2O \longrightarrow 5HCOOH + 8Ce_2(SO_4)_3 + 8H_2SO_4$$

The presence of the formic acid in the oxidation products can readily be demonstrated by steam distillation and reduction of mercuric chloride by the steam distillate; previous workers[1,2,3] have observed that oxidation of organic compounds by the ceric sulphate procedure often results in the formation of formic acid.

The method is as follows.

SOLUTIONS REQUIRED—(1) Ceric sulphate solution, 0·1 *N*. (2) Ferrous ammonium sulphate solution, 0·1 *N*. (3) Sulphuric acid solution, 6 *N*. (4) N-Phenyl anthranilic acid indicator solution: dissolve 0·0533 g. of N-phenyl anthranilic acid in a solution of 0·05 g. of Na_2CO_3 in 5 ml. of water, and dilute the solution to 50 ml. with distilled water.

PROCEDURE—To 10 ml. of the solution under examination add 50 ml. of 0·1 *N* ceric sulphate solution, 20 ml. of 6 *N* sulphuric acid solution and 50 ml. of water and boil the mixture under reflux in an all-glass apparatus for 1 hour and then cool. Titrate the cold solution with 0·1 *N* ferrous ammonium sulphate solution until a slight excess is present, and determine this excess by titration with 0·1 *N* ceric sulphate solution, using two drops of N-phenylanthranilic acid indicator solution. The end-point change is from yellow to rose madder.

Make a blank determination on 50 ml. of the 0·1 *N* ceric sulphate solution under corresponding oxidation conditions. It is desirable that in the determination itself not more than one half of the ceric sulphate should be used up in the oxidation.

1 ml. of 0·1 *N* ceric sulphate solution $\equiv$ 0·000638 g. of tetrahydrofurfuryl alcohol.

The following results have been obtained on oxidation of known amounts of tetrahydrofurfuryl alcohol.

Tetrahydrofurfuryl alcohol taken, g.	0·147	0·0065	0·0017
" " found, g.	0·148	0·0063	0·0017

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IMPERIAL CHEMICAL INDUSTRIES LTD.
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J. HASLAM
L. H. RUDDLE

February, 1949

THE DETERMINATION OF ETHYL ALCOHOL IN ETHYL ETHER

SOME technical supplies of ether used during the war for the extraction of propellant mixtures were found to contain as much as 0·5 to 1·0 per cent. of ethyl alcohol as impurity and it was found that this was not removed by the usual treatment with potassium permanganate and potassium hydroxide followed by drying and distillation. The impurities present in this ether were isolated by careful re-fractionation and shown to consist of ethyl alcohol together with a trace of acetone and of mineral jelly.

Since small quantities of ethyl alcohol in supposedly pure ether may cause variable solubility losses when nitrocellulose is determined as the residue after prolonged extraction with ether, it was important to detect and determine the amount of alcohol present and to ensure its complete removal.

In the determination of acetone in ether by Messinger's method[1] it has been shown that, whilst the method is satisfactory for acetone even at high dilution, no reaction occurs with ethyl alcohol at similar concentrations and the presence of this impurity is not disclosed. An additional determination of the alcohol content of ether was therefore necessary and one that has been found to be particularly suitable depends on the formation of a red co-ordination complex between alcohols and ceric ammonium nitrate solution.[2] A method based on this reaction is described by F. Duke,[3] who recommends the use of a photo-electric spectrophotometer to measure the intensity of the colour produced, and corrects for the fading caused by oxidation of the alcohol by extrapolating to zero time. However, the determination may be made more simply and rapidly by the method described below, requiring only the simplest apparatus. A single determination can be made in a very few minutes with an accuracy quite adequate for the purpose, the effect of fading being minimised by the short period required for the matching of the colour produced.

It should be noted that the colour is given by all the lower alcohols and that aldehyde, amines, etc. interfere.[3]

METHOD—

To prepare the ceric ammonium nitrate solution, dissolve 667 g. of ceric ammonium nitrate, $(NH_4)_2Ce(NO_3)_6$, in the minimum quantity of water and make up the total volume to 1 litre. Filter the solution if cloudy. This reagent will keep for an indefinite period.

Extract 50 ml. of the ether to be tested in a separating funnel with three 15-ml. portions of water. Place the combined extract in a 50-ml. Nessler cylinder. Into a second cylinder measure 40 ml. of distilled water and 5 ml. of the reagent. Finally, add 5 ml. of the reagent to the cylinder containing the aqueous extract and rapidly titrate the contents of the second cylinder with a 3 per cent. (w/v) solution of alcohol in water. When the colour of the blank almost matches that in the original tube adjust the volume of liquid in the two tubes to the same level before completing the titration. When the colour matches

the alcohol content of the ether, g./100 ml. = ml. of 3% alcohol required × 0·06
and " " " " " g./100 g. = ml. of 3% alcohol required × 0·06/0·720.

This method was used to determine the alcohol content of known ether - alcohol mixtures with the result shown below.

TABLE I

Alcohol present in ether, g./100 ml.	Titre of 3% alcohol ml.	Alcohol in ether, determined g./100 ml.
0·10	1·65	0·099
"	1·70	0·102
"	1·65	0·099
"	1·65	0·099
"	1·60	0·096
0·20	3·20	0·192
"	3·30	0·198
"	3·30	0·198
"	3·25	0·195

As will be seen from Table I the extraction of alcohol is complete under the conditions of the method and subsequent estimation of quantities between 0·10 and 0·20 per cent. is satisfactory. The end-point may be judged to within 0·1 ml., corresponding to an error of 3 per cent. on 0·10 per cent. of alcohol.

The method will detect the presence of 0·01 per cent. of alcohol.

PREPARATION OF ALCOHOL-FREE ETHER—

Ether may be rendered completely free from alcohol by washing with a 10 per cent. solution of sodium chloride. One litre of ether can be conveniently treated in a 2-litre separating funnel with three successive 300-ml. portions of salt solution. The solubility of ether in 10 per cent. salt solution is small and the loss of solvent on this account is negligible.

The alcohol-free ether is dried and distilled in the usual way.

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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CHEMICAL INSPECTORATE
MINISTRY OF SUPPLY

J. LAMOND
April, 1949

A NEW METHOD FOR THE DETECTION OF SEMI-MICRO QUANTITIES OF ZINC IN COBALT SALTS

FEW methods are available for the detection of small amounts of zinc in cobalt salts. Removal of cobalt by the addition of alkali always leads to excessive co-precipitation of zinc. Most of the methods available are only of use when the zinc/cobalt ratio is of the order 1/1000, and two polarographic methods have been described[1,2] that give a means of separation of the two elements provided that cobalt is not in great excess. An accurate but lengthy dithizone separation may be used[3] but the procedure described below serves as the basis for a rapid limit test which will detect 50 p.p.m. of zinc in cobalt salts. The procedure is similar to that described by Miller[4] but the separation of the zinc depends on the greater solubility of zinc thiocyanate in ether. Part of the cobalt is also extracted as the thiocyanate but the bulk is retained in the aqueous phase. The small amount of cobalt that is extracted with the zinc can be removed from active interference by conversion to potassium cobalticyanide, which then serves as a precipitant for zinc in acid solution.

METHOD—

Dissolve 5 g. of cobalt chloride, nitrate or sulphate in 20 ml. of water, add 1 ml. of 5 *N* hydrochloric acid and 5 ml. of 7·5 *M* ammonium thiocyanate and extract with 15 ml. of ether, shaking vigorously. Evaporate the ether from the ethereal extract and to the residue add cautiously 0·5 ml. of nitric acid (sp.gr. 1·42). When the vigorous reaction has ceased, evaporate to dryness, dissolve the residue in 1 drop of 5 *N* hydrochloric acid and 4 ml. of water, boil and filter. To the filtrate add 1·5 *M* potassium cyanide solution drop by drop until the precipitate formed just redissolves. Pass a current of air through the solution for 5 minutes, add 2 drops of hydrochloric acid (sp.gr. 1·18) and allow to stand for 1 hour. In presence of 0·25 mg. of zinc a turbidity will be formed.

Thanks are due to the Directors of Hopkin & Williams Limited for permission to publish this work.

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E. A. JOHNSON
W. C. JOHNSON
April, 1949

Ministry of Food

CIRCULAR MF15/49

(England and Wales)

This circular, dated August 31st, 1949, of which the following is a summary, draws attention to
The Milk (Special Designation) (Raw Milk) Regulations, 1949,
The Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations, 1949,
(see below), which re-enact with amendments the provisions of the Milk (Special Designations) Regulations, 1936 to 1948. The major changes introduced in these regulations are consequent upon the relevant provisions of the Food and Drugs (Milk and Dairies) Act, 1944 (which comes into operation on October 1st, 1949) as amended by the Food and Drugs (Transfer of Functions) Order, 1948, and the Milk (Special Designations) Act, 1949. Other changes of detail which experience has shown to be desirable and, in some particulars, necessary have been made.

4. For the time being the use of special designations in relation to milk will remain a voluntary act on the part of the person or council concerned; in due course, however, when areas have been specified under the Milk (Special Designations) Act, the position will be radically different in as much as the use of special designations in specified areas will be obligatory upon all who wish to sell milk by retail in such areas. Local authorities and Food and Drugs Authorities concerned with the execution and enforcement of the regulations in specified areas will have added responsibilities as the agents of the central government for carrying into effect the policy which has been given legislative form in the Milk (Special Designations) Act, 1949.

Paragraphs 5 to 9 of the Memorandum are concerned with provisions relating to dealers' licences.

Paragraphs 10 to 12 relate to the Milk (Special Designation) (Raw Milk) Regulations. The designations authorised by these regulations are, as at present, "Accredited" and "Tuberculin Tested." The use of the former designation is, however, now limited to a period of five years from the commencement of these regulations, *i.e.*, until October 1st, 1954; in addition, it should be noted that section (2) of the Milk (Special Designations) Act restricts the use of the designation "Accredited" in specified areas to milk derived from a single herd.

Paragraphs 13 to 16 relate to the Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations. The designations authorised under these regulations are "Pasteurised" and "Sterilised"; the latter is a new designation and has been incorporated into the regulations in anticipation of the time when areas are specified and it will be necessary to legalise in them the sale of sterilised milk.

14. *Licensing authorities*—Responsibility for the granting of dealers' (pasteurisers' and sterilisers') licences has been transferred from local authorities to Food and Drugs Authorities who are responsible for the execution and enforcement of many allied provisions of the Milk (Special Designations) Act.

15. *General and special conditions subject to which licences are granted*—The general and special conditions laid down in the schedules to these regulations are additional to any relevant provisions of the Milk and Dairies Regulations and of the Milk (Special Designation) (Raw Milk) Regulations. The regulations include details of the pasteurising and sterilising processes, they provide for both the Holder and the Higher Temperature Short Time processes and in addition give the Authority, with the Minister's approval, discretionary power to approve any other time and temperature, a provision which will enable the Authority, with the Minister's approval, to keep in step with new methods of heat treatment which tests have shown to be satisfactory. The Minister, on the advice of the best authority available, has reduced the minimum temperature under the H.T.S.T. process by 1° F. Under properly operated conditions there is, on present evidence, no risk to health, but the change will result in an improved "cream line" on the milk and will doubtless help to popularise pasteurised milk among consumers. The Authority will note that as from October 1st, 1950, until October 1st, 1954, milk which is pasteurised in bottles (in-bottle pasteurisation is provided for in the regulations) shall be delivered to the consumer in those bottles, and milk which is pasteurised by the usual method in other containers shall be put into the bottles or other containers in which it is to be delivered to consumers only by the person holding a licence to use the designation "Pasteurised"

in relation to such milk and on registered premises named in that person's licence. As from October 1st' 1954, all pasteurised milk shall be put into bottles or other containers in which it is to be delivered by retail on the premises on which it is pasteurised.

16. Both the Raw Milk Regulations and the Pasteurised and Sterilised Milk Regulations make provision for keeping designated milk apart from all other milk. In each case the dealers' arrangements must be adequate to ensure that the milk to which the licence applies is not contaminated by any other milk. The Minister is aware that the corresponding provision in the Milk (Special Designations) Regulations, 1936 to 1948, has given rise to difficulty and variation of interpretation, with the result that, in some districts, Tuberculin Tested milk is being handled in small and congested premises and the main object of the provision is in danger of being defeated. The present regulations have a proviso contained in paragraph 2 of the First Schedule, which has been inserted with the object of assisting licensing authorities in the execution and enforcement of the condition. The Minister sees no objection to undesignated milk and designated milk or to two or more kinds of designated milk being handled, etc., on the same premises provided that adequate arrangements are made to prevent the milk to which the designation applies being contaminated by any other milk, and he is of the opinion that the cleansing of vessels and plant can be dispensed with when the circumstances are such as to exclude any possibility of contamination of the designated milk by any other milk. The use of vessels and plant for handling Accredited milk *immediately* after such vessels and plant had been used for handling Tuberculin Tested milk would, in his opinion fall into this category; on the other hand, the handling of pasteurised milk immediately after raw milk (whether designated or not) should not be permitted, as in such circumstances there may be some risk of contamination of the pasteurised milk by the unpasteurised milk.

Ministries of Food, Health, and Agriculture and Fisheries

STATUTORY INSTRUMENTS*

1949.—No. 1588. The Milk and Dairy Regulations, 1949. Pp. 17. Price 6d.

These Regulations, issued jointly by the Ministry of Health, the Ministry of Agriculture and Fisheries and the Ministry of Food, came into operation on October 1*st,* 1949. *They re-enact with amendments the Milk and Dairies Regulations,* 1926 *to* 1943.

The principal changes are consequent upon the provisions of the Food and Drugs (Milk and Dairies Act), 1944, *and the Agriculture (Miscellaneous Provisions) Act,* 1949.

The Minister of Agriculture and Fisheries thus becomes responsible for the registration of dairy farms and dairy farmers and for the enforcement of the regulations on dairy farms (except in so far as they relate to diseases communicable to man).

The local authorities retain responsibility for those provisions which apply outside dairy farms, for the provisions relating to diseases communicable to man and for the registration of dairies that are not dairy farms and of dairymen who are not dairy farmers.

A Central Milk and Dairies Advisory Committee and County Milk and Dairies Advisory Committee are set up to review and make recommendation as to the operation of these Regulations and the Milk (Special Designation) Regulations.

New provisions are made for the inspection of cattle on dairy farms by veterinary inspectors of the Ministry of Agriculture and Fisheries.

There are a number of minor changes relating to buildings, the cleansing of vessels and utensils and the distribution of milk and its protection against contamination and infection and, in particular, provision is made for modern practices in dairying, including the use of mechanical refrigeration for cooling and of approved chemical agents for cleansing apparatus and appliances.

— No. 1589. The Milk (Special Designation) (Pasteurised and Sterilised Milk) Regulations. Pp. 18. Price 6d.

These Regulations made jointly by the Ministry of Health and the Ministry of Food came into operation on October 1*st,* 1949. *They revoke and re-enact with amendments the Milk (Special Designations) Regulations,* 1936 *to* 1948 *(S.R. & O.,* 1936, *No.* 356; 1938, *No.* 218; *P.R. & O., July* 9*th,* 1941; *S.R. & O.,* 1942, *No.* 771; 1943, *No.* 1645; 1946, *No.* 10; *and S.I.,* 1948, *No.* 1117), *so far as they relate to pasteurised milk and provide for a new special designation, "sterilised milk."*

The principal changes are consequent upon the provisions of the Food and Drugs (Milk and Dairies Act, 1944, *and the Milk (Special Designations) Act,* 1949. *In accordance with the first-mentioned Act, as modified by the Transfer of Functions (Food and Drugs) Order,* 1948, *regulations dealing with the special designations of raw milk are made jointly by the Minister of Health, the Minister of Agriculture and Fisheries and the Minister of Food, and are contained in separate regulations, the Milk (Special Designation) (Raw Milk) Regulations,* 1949. *These new regulations are made jointly by the Minister of Health and the Minister of Food; they are, therefore, concerned only with the special designations of heat-treated milk.*

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* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

The special designations which may be used in accordance with these regulations in relation to milk are "Pasteurised" and "Sterilised."

The regulations provide also for the use of the special designations "Tuberculin Tested Milk (Pasteurised)" and "Tuberculin Tested Milk (Sterilised)."

The conditions under which milk shall be pasteurised or sterilised are closely defined and the provisions for sampling laid down.

The procedures for the phosphatase and methylene blue tests for pasteurised milk, and the turbidity test for sterilised milk are described in full detail (pp. 4).

— **No. 1590. The Milk (Special Designation) (Raw Milk) Regulations.** Pp. 21. Price 6d.

These Regulations made jointly by the Ministry of Health, the Ministry of Agriculture and Fisheries and the Ministry of Food came into operation on 1st October, 1949. They revoke and re-enact with amendments the Milk (Special Designations) Regulations, 1936 to 1948, so far as they relate to raw milk (the revoked orders are the same as those noted under Regulation No. 1589, above).

The special designations authorised by these regulations which may be used in relation to milk are "Tuberculin Tested" and "Accredited."

After October 1st, 1957, the special designation "Tuberculin Tested" may only be used in respect of milk from a herd which is on the Register of Attested Herds kept by the Minister of Agriculture and Fisheries.

After October 1st, 1954, the special designation "Accredited" will no longer be permitted.

These regulations provide that milk sold under a special designation shall satisfy a prescribed methylene blue reduction test.

Provisions as to sampling, the transport and keeping of samples, and identification of samples are laid down.

The procedure to be followed in the methylene reduction test and its interpretation are described in full detail.

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, J. H. Lane, 7–8 Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel.

CK(ISE) 5633—Draft on the Determination of Chromium in Ferro-Chromium (Superseding CK(ISE) 4375).

Draft Specification prepared by Technical Committee OSC/21—Marine, Animal and Fish Oils.

CK(OSC) 6427—Draft for Crude Sperm Oil (Revision of B.S.997—1941).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Biochemical

Colorimetric Determination of Thyroxine. D. Winikoff and V. M. Trikojus (*Biochem. J.*, 1948, **42**, 475–480)—The method is based on the colour formed by treating thyroxine with N^1-diethylsulphanilamide in alkaline solution.

REAGENTS—(1) *N^1-diethylsulphanilamide*—Dissolve 40 g. of 4-acetamido-benzenesulphonyl chloride in 200 ml. of acetone and add this solution to a cold aqueous solution of diethylamine prepared from 44 g. of diethylamine hydrochloride and 200 ml. of 2 *N* sodium hydroxide. Cool and add 16 g. of powdered sodium hydrogen carbonate. Keep at room temperature for some hours, shaking frequently. Remove the acetone by heating under reduced pressure on a water-bath, chill the residue, and add 200 ml. of water. Boil the precipitate for a few minutes in 40 ml. of 6 *N* hydrochloric acid. Add water and heat for a few minutes. Cool the hot filtered solution and make it alkaline with 2·5 *N* sodium hydroxide. Wash the resulting precipitate with water and re-crystallise first from 60 per cent. ethanol with the aid of charcoal and then from a mixture of ethanol and light petroleum (b.p. 60° to 80° C.). The product should be colourless prisms, m.p. 105° to 106° C. *Diazotisation*—Dissolve 1·14 g. of the amide in 9 ml. of 10 *N* hydrochloric acid. Dilute to 100 ml. with water. Take 5 ml. of this solution and, keeping it at 0° C., add 5 ml. of 4·5 per cent. (w/v) sodium nitrite solution. Add 20 ml. of ice-cold water, and use the reagent after 10 min. standing.

(2) n-*Butanol*—Purify by distillation over solid sodium hydroxide and use the fraction boiling at 116° C.

PROCEDURE—Use 2 ml. of a solution containing 1 to 5 mg. of thyroxine per 100 ml. in 0·1 *N* sodium hydroxide. Place in a glass-stoppered, 15-ml. centrifuge tube, add 2 ml. of *n*-butanol and then 1 ml. of 2 *N* sodium hydroxide. Mix by shaking, but do not let the liquid reach the neck of the tube. Add 3 ml. of a solution containing 1 ml. of 2 *N* sodium hydroxide and 2 ml. of the diazonium solution. Stopper the tube and shake well for 15 sec. Set aside for 5 min., remove the stopper, and then keep the tube for 24 hr. on a gently-shaken rack in a thermostat at 25° C. Also place on the rack a control tube containing 2 ml. of 0·1 *N* sodium hydroxide plus reagents.

Stopper the tubes and centrifuge for 30 min. at 2500 r.p.m. Transfer the butanol phase with a Pasteur pipette to a small dry tube. To 0·75 ml. of this solution add 0·25 ml. of *N* alcoholic sodium hydroxide to prevent the formation of a turbidity. The solution can be stored for 24 hr. if evaporation of solvent and access of carbon dioxide are prevented. Determine the absorption with a Hilger Spekker absorptiometer, using the Ilford green filter (maximum absorption is at 540 mμ.). Prepare a calibration curve based on pure thyroxine.

A preliminary treatment of some biological material may be required. Treat with ether before extracting the acid-insoluble thyroxine precipitate with butanol or else extract the thyroxine into aqueous alkali from the butanol extract by adding light petroleum. Re-extract the aqueous phase with butanol and continue as above.

W. S. Wise

Determination of 4-Methyl-2-Thiouracil in Animal Tissue and Blood. H. van Genderen, K. L. Van Lier, and J. De Beus (*Biochim. et Biophys. Acta,* 1948, **2**, 482–486)—Difficulties encountered in the determination of low concentrations (*e.g.*, 1 mg. per 100 g.) of 4-methyl-2-thiouracil by previous methods for the determination of thiouracil (Williams *et al.*, *J. Clin. Invest.*, 1944, **23**, 613; *J. Lab. Clin. Med.*, 1944, **29**, 329) were due not to the lack of sensitivity of the colour reaction with Grote's reagent, but to the method of precipitation. The recovery of added methylthiouracil was poor, and red-coloured reaction products of Grote's reagent with impurities were formed.

From a study of the effects of pH and the distribution of methylthiouracil between different buffer - organic solvent systems along the lines indicated by Morch (*Acta Pharmacol. Toxicol.*, 1945, **1**, 106), a routine experimental procedure has been evolved.

The purification of a chloroform - ethanol (20 per cent.) extract of the acidified and dried blood or tissue pulp, by partition between an acid aqueous phase and chloroform (without ethanol), makes possible constant, high recoveries of added methylthiouracil. With a buffer of pH 8 almost all the methylthiouracil remains in the aqueous phase.

In presence of ethanol, the development of the colour with Grote's reagent is slower, but the colour is very stable.

Method—*Preparation of Grote's reagent*—Dissolve 1 g. of sodium nitroprusside and 1 g. of hydroxylamine hydrochloride in 20 ml. of water, add 2 g. of sodium bicarbonate, shake until the effervescence stops, add 0·1 ml. of bromine, and shake again. Filter the solution into a volumetric flask and dilute with water to 50 ml. The reagent can be kept in the ice-box for about 10 days. Variations between different batches of the reagent are difficult to avoid, and it is therefore necessary to conduct an experiment with a standard solution of methylthiouracil once a day.

Colorimetric reaction—Adjust the extract of serum, blood or tissue to a pH of from 7·9 to 8·1 with a boric acid - borax buffer solution of pH 8·0. Add 10 per cent. of 96 per cent. ethanol to the extract, followed by 0·4 ml. of Grote's reagent per 10 ml. of extract, and allow the mixture to stand at room temperature for 30 min. Measure the light extinction in a spectrophotometer at 6650 A. in a 3-cm. cell. The Beer - Lambert law holds for the extinction - concentration curve over a wide range.

Precipitation method for serum—Add 5 ml. of a freshly prepared mixture of equal parts of 10 per cent. sodium tungstate solution and 0·66 *N* sulphuric acid to 5 ml. of blood serum in a centrifuge tube. Shake the tube vigorously and centrifuge. Take 5 ml. of the clear supernatant fluid and add 2 ml. of borate buffer (pH 8·0) and 1 ml. of ethanol. Adjust the pH of the sample to 8·0 and make the final volume 10 ml. Of 1 mg. of methylthiouracil added to the serum per 100 ml., 82 to 88 per cent. can be recovered.

Extraction procedure for blood and tissue—Grind 10 g. of tissue in a mortar with sand and add 7 to 10 ml. of 0·5 *N* alcoholic hydrochloric acid, to bring the pH of the material to below 4. Transfer the material to a distilling flask with a few millilitres of ethanol and evaporate the extract to dryness under reduced pressure on a water-bath at 70° C. Repeat the evaporation after addition of 10 ml. of methanol. For blood, pipette 10 ml. of blood directly into the distilling flask, add 10 ml. of 0·5 *N* alcoholic hydrochloric acid and, after mixing, follow the evaporation procedure described for tissue. Disperse the dried tissue or blood cake with 10 ml. of ethanol and add 50 ml. of chloroform or of methylene chloride. Heat under refluxing conditions for 15 min. on a water-bath and decant the liquid through a filter into a distilling flask. Repeat twice the extraction with the chloroform - ethanol mixture, combine the three extracts and filter. Evaporate the filtrate to dryness on the water-bath. With successively 5 ml., 3 ml., and 2 ml. of chloroform (or methylene chloride) and 3 ml. of distilled water transfer the residue to a centrifuge tube. Transfer most of the clear water layer to a tube and extract the chloroform with two more 3-ml. portions of water. Combine the three aqueous extracts, adjust the pH to 8 by means of borate buffer and sodium hydroxide solution. Add 1·5 ml. of ethanol before filtering through a hard filter if necessary. Dilute to a volume of 15 ml. with the buffer solution and add 0·6 ml. of reagent. Continue the determination as previously described.

Results—Blank determinations on blood or tissue without methylthiouracil usually give extinction readings slightly higher than those of aqueous "blanks." The colour is yellow, but light red or brown discolorations, which do not necessarily interfere with the measurement of the green reaction product of methylthiouracil, may appear.

For a single determination in the range of 100 µg. of methylthiouracil per 10 g. of tissue the result is expected to have an error not greater than ±6 µg. G. A. STEWART

Use of Sodium Hypochlorite in the Determination of Alanine, Valine, and Leucine. E. Aubel and J. Asselineau (*Biochim. et Biophys. Acta*, 1948, **2**, 198–206)—The general reaction and the method for alanine are dealt with in a previous paper (*Idem., Bull. Soc. Chim.*, 1947, 114 M; *Analyst*, 1948, **73**, 693).

Determination of valine and leucine in a mixture of the two—The process involves the separation of *iso*butyraldehyde (b.p. 63° C.) and *iso*valeraldehyde (b.p. 92° C.). The aldehydes are therefore entrained from the reaction flask into carbon tetrachloride, which has an intermediate boiling-point (76° C.), and the *iso*butyraldehyde is then separated by the use of a 1·5-metre fractionating column packed with glass rings and jacketed with a water-cooled condenser on the upper portion. The boiling-flask has an air inlet tube that passes into the liquid, and the head of the column consists of a swan-neck leading into a trap containing potassium bisulphite solution. The *iso*butyraldehyde concentrates at the top of the column and is entrained during 1·5 hr. into the bisulphite by a suitably regulated air current. The excess of bisulphite is titrated with iodine in the usual manner. Results are up to 5 per cent. high and results for *iso*valeraldehyde, which are obtained by difference, are correspondingly low.

Analysis of mixtures of alanine, valine, and leucine—When alanine is also present the acetaldehyde produced is entrained with the *iso*butyraldehyde during the fractionation. The acetaldehyde is then determined by the colorimetric method (*loc. cit.*). Valine + alanine are determined iodimetrically, and valine by difference. Leucine is also calculated by difference after straight entrainment of all three aldehydes followed by iodine titration. (In the example quoted, alanine is determined in the mixture of the *three* aldehydes.) In a table of results for various mixtures of the three amino acids the maximum errors range from +6 to −4·5 per cent. for alanine, from +4 to −7·6 per cent. for valine, and from +6·7 to −15 per cent. for leucine.

Determination of valine in protein hydrolysates—When applied to a complex mixture of amino acids the usual process of air entrainment leads to low results for valine and leucine; steam distillation is therefore adopted. Under these conditions the aldehydes derived from glycine, phenylalanine, and methionine are also entrained; consequently leucine cannot be determined by difference. *Procedure*—Decompose the chloroamino compounds by adding the reaction mixture during 15 to 20 min. to 60 ml. of boiling 0·1 *M* phosphate buffer, receiving the distilled aldehydes in 10 ml. of water cooled in ice. Transfer the receiver to a Fuchs apparatus (*Z. physiol. Chem.*, 1933, **221**, 271), heat the solution to boiling, maintain an air current of 500 ml. per min., and receive the aldehydes in carbon tetrachloride cooled in an ice-salt mixture. This operation requires 15 min. Fractionate the carbon tetrachloride solution of aldehydes, determine the total aldehydes in the distillate by iodine titration, the acetaldehyde colorimetrically, and the *iso*butyraldehyde, and thence the valine, by difference.

Determination of leucine in a complex mixture of amino acids—The mixture considered contains 3 to 6 mg. each of leucine, alanine, valine, glycine, aspartic acid, phenylalanine, and methionine. The solution is passed through a column of 5 g. of "acid" alumina (Asselineau, *Bull. Soc. Chim.*, 1947, 1065 M), concentrated under reduced pressure, adjusted to pH 6·5 to 6·8, and passed through a column of activated silver sulphide (Hamoir, *Biochem. J.*, 1945, **39**, 485). The usual hypochlorite treatment is applied. The formaldehyde derived from the glycine is retained by the addition of asparagine to the phosphate buffer (Peynaud, *Bull. Soc. Chim.*, 1946, **40**, 685). The distillate contains only the aldehydes derived from leucine, valine, and alanine. The analysis is completed according to the principles already described.

W. C. JOHNSON

Determination of Copper and Lead in Biological Material. P. R. v. d. R. Copeman (*Union of South Africa, Dept. of Agric., Science Bulletin No.* 251)—Satisfactory procedures have been evolved from well known methods for the determination of these elements in biological material.

Preparation of the sample—Treat a known weight of material (not more than 50 mg.) with 100 ml. of 10 per cent. nitric acid in a Kjeldahl flask, gradually bringing the solution to the boiling-point, until the material is completely disintegrated. If any undissolved material remains, filter it off and treat it with a small amount of nitric acid in another Kjeldahl flask. Add 10 to 15 ml. of concentrated sulphuric acid to the combined solutions and evaporate, adding a small amount of concentrated nitric acid to the hot solution when it begins to darken. When a clear solution remains and white fumes appear, cool, and add 20 ml. of water or of a 10 per cent. solution of ammonium oxalate. Evaporate again until white fumes appear, cool and dilute the solution to a known volume in a volumetric flask.

Procedure for the determination of copper—To 5 ml. of a solution representing 50 mg. of tissue in 50 ml. add 5 ml. of a 20 per cent. solution of citric acid and 2 ml. of a 4 per cent. solution of sodium pyrophosphate. Make the solution alkaline to litmus with concentrated aqueous ammonia solution adding 0·5 ml. in excess, cool, and add 2·5 ml. of carbon tetrachloride followed by 1 to 2 ml. of a 2 per cent. aqueous solution of sodium diethyldithiocarbamate. Shake the mixture well, and after allowing the liquids to separate, run off the golden-yellow carbon tetrachloride solution into a dry measuring cylinder. Repeat the extraction with further 2·5-ml. portions of carbon tetrachloride until no further colour is extracted. Dilute to a known volume with carbon tetrachloride and compare the colour with that of a standard prepared in the same way. It is necessary to carry out a blank determination and apply the appropriate correction.

The carbon tetrachloride extract must be free from water and, if necessary, it can be dried by adding a small amount of anhydrous sodium sulphate and shaking. It should not be filtered through a filter paper, since such paper usually contains traces of copper. Using the above procedure, no substance normally interferes. Excellent recoveries are obtained for determinations on various animal tissues as well as on materials such as paper, cereals, and salt.

Procedure for the determination of lead—Transfer a portion of the solution (usually 25 ml.) to a separating funnel, add 5 ml. of a 20 per cent. solution of citric acid, and make the solution alkaline to litmus with concentrated aqueous ammonia solution, adding a slight excess. Cool, add 2 ml. of a freshly prepared 10 per cent. solution of potassium cyanide and extract with 5 ml. of a 0·05 per cent. solution of dithizone in chloroform. (If the volume of the original solution is greater than 25 ml., a correspondingly larger volume of potassium cyanide solution must be added.) Allow the liquids to separate and run the chloroform layer into a clean tube. Continue to extract the aqueous solution with the dithizone solution until the chloroform layer is no longer red, but green, finally washing with pure chloroform. Transfer the combined extracts to a test tube, and distil off the chloroform. Add 1 ml. of concentrated sulphuric acid and 1 ml. of concentrated nitric acid and heat the contents of the tube over a small flame, adding two glass beads to minimise bumping. Add a few drops of nitric acid at intervals to obtain a colourless solution, and continue to heat until white fumes appear. Cool, add 10 ml. of 50 per cent. ethyl alcohol, and allow the solution to stand overnight while the lead sulphate precipitates. Filter the solution through a tightly packed pad of filter paper with the aid of suction, and wash the test tube and pad with 10 to 15 ml. of 50 per cent. ethyl alcohol acidified with a few drops of concentrated sulphuric acid. Dissolve the lead sulphate in a 20 per cent. solution of ammonium acetate, and pour two 5-ml. portions of warmed ammonium acetate through the pad. Wash the pad with 5 ml. of warm water and dilute the combined solution to 25 ml. Transfer 5 ml. of the final solution to a 50-ml. separating funnel, add 2 drops of phenol red indicator solution, and sufficient aqueous ammonia solution to give a red colour. Add 1 ml. of a 10 per cent. solution of potassium cyanide and then add a 0·005 per cent. solution of dithizone in chloroform in small amounts from a burette. After each addition, shake the mixture, allow it to settle and run off the red chloroform layer. When the chloroform layer shows a purple tint, reduce the volume of the additions of dithizone solution and wash the aqueous solution with pure chloroform, leaving a few drops in the funnel each time. The titration is complete when the chloroform solution remains green after the addition of dithizone. A blank determination must be carried out and the necessary correction applied.

Satisfactory results have been obtained for the determination of lead in various tissues, as well as in urine and in paper, cereals, and salt.

J. G. WALLER

Biochemistry of Scandium and its Separation as Phytate. G. Beck (*Mikrochem.*, 1948, **34**, 62–66)—Although scandium is more abundant than many more familiar elements (*e.g.*, 10 times more than iodine) it has been little studied. Its wide distribution and availability to plants has prompted this investigation of methods of detection in the animal organism.

The known affinity of scandium for the phosphate ion led to the use of phytic acid (inositol hexaphosphoric acid) as precipitant. Scandium phytate, $Sc_6C_6H_6P_6O_{27},36H_2O$, is obtained as a white flocculent precipitate of very stable constitution and is insoluble in concentrated hydrochloric acid. It is unaffected by nitric acid, *aqua regia*, and hydrogen peroxide even at the boiling-point. A solution of scandium containing 30 μg. of the element per ml. and acid with hydrochloric acid yields a turbidity on addition of sodium phytate. Titanium, zirconium, hafnium and thorium also yield acid-insoluble precipitates, but zirconium and hafnium phytates dissolve in oxalic acid. Zirconium, hafnium, and titanium phytates are also soluble in alkali fluoride, whilst thorium phytate dissolves in cyanacetic acid. Scandium phytate is stable towards alkali and towards ammonium carbonate; it dissolves in oxalic acid solution buffered with sodium acetate, but only over a narrow pH range. The phytates of aluminium and of the rare-earth metals are soluble in dilute mineral acids but not in acetic acid or in ammonium carbonate. Aluminium phytate is also soluble in alkali hydroxide. The gallium compound is the only phytate mentioned that is soluble in aqueous ammonia. Beryllium and indium phytates are somewhat soluble in ammonium carbonate.

Detection of scandium in urine—Evaporate 6 litres of urine in a porcelain basin, decompose the organic matter with nitric acid, and dissolve the residue in dilute nitric acid. Filter off the carbon particles, ignite them with potassium nitrate in a platinum dish, dissolve the mass in nitric acid and add to the main solution. Add 1 ml. of a 30 per cent. solution of sodium phytate and a drop of ferric chloride solution. If necessary reduce the acidity by adding aqueous ammonia solution. The precipitated ferric phytate carries down scandium and zirconium. Centrifuge, wash the sediment, fuse it with a small amount of sodium hydroxide with addition of some potassium nitrate, and leach out with water (Solution 1). Dissolve the residual ferric hydroxide in hydrochloric acid, precipitate the iron by ammonia, add a few drops of ammonium sulphide, and boil with potassium cyanide until all the iron goes into solution. Add ammonium chloride to destroy alkali carbonate and centrifuge the trace of white residue. Boil Solution 1 with ammonium chloride for a considerable time and then centrifuge it with the residue obtained from the dissolution of the ferric hydroxide. Boil [the combined residues, presumably] with hydrochloric acid and centrifuge from traces of silicic acid. Neutralise 3 ml. of the clear solution with ammonia, add ammonium carbonate, and filter from precipitated aluminium hydroxide. Place one or two drops of the filtrate on a microscope slide and add a drop of saturated sodium fluoride solution and a few granules of hexamminocobaltic chloride or nitrate. After 5 min. observe under the microscope with high magnification. Scandium forms yellowish cubes of $[Co(NH_3)_6]ScF_6$ (*Idem., Mikrochim. Acta*, 1937, **2**, 9). Mix another portion with sodium phytate; scandium yields a turbidity unaffected by hydrochloric acid or oxalic acid. The estimated quantity of scandium is usually 10 to 20 µg. per litre (limit of detection with phytate). No reaction for titanium is obtained with hydrogen peroxide. Treat another portion of the solution with a very little alizarin sulphonic acid solution. The resulting red colour persists on addition of hydrochloric acid but disappears with sodium fluoride. Sodium phosphate causes a slight precipitate insoluble in hydrochloric acid. These reactions indicate zirconium in µg.-quantities.

Scandium in milk—Two litres of milk treated similarly showed a scandium content similar to that of urine.

Scandium is also found in ox blood to the extent of 50 to 100 µg. per litre, in pig's liver in similar concentration, and about 10 µg. per kg. in the human brain.

Scandium can also be used as a reagent for phytic acid. A solution of the latter, containing 300 µg. per ml., gives with scandium sulphate a turbidity that does not clear on adding a drop of dilute sulphuric acid. W. C. JOHNSON

Micro-polarographic Determination of Chloride Ions in Biological Fluids. F. Santavy (*Coll. Czech. Chem. Comm.*, 1948, **13**, 557–560)—The anodic wave given by chloride ions can be used for the polarographic determination of chloride in biological material. The method has the advantage that albumens need not be removed and also that it can be carried out rapidly on small volumes.

Procedure—Make the solution to be examined 0·1 *N* with respect to sulphuric acid, remove dissolved oxygen by passing a stream of inert gas through the solution, and record a polarogram from +0·4 to 0 v. *versus* the mercury pool. The chloride ion concentration in the final solution should not exceed 0·004 *N*.

Determinations on a number of biological fluids, using as little as 1 cu. mm. of liquid, gave results in good agreement with those obtained by other methods. J. G. WALLER

Organic

Electric Furnace for Semi-micro-analysis of Carbon and Hydrogen. H. E. Ungnade and K. Zilch (*Anal. Chem.*, 1948, **20**, 996)—The copper oxide filling of commercial micro-furnaces is inadequate with larger samples, 15 to 25 mg., but a longer copper oxide section and correspondingly longer furnace give excellent results. A 33-cm. furnace (Hevi Duty Electric Co.) has been modified by using a Pyrex 172 micro-combustion tube about 11 mm. in outside diameter resting in a stainless steel or iron pipe about 12 mm. in inside diameter, held in place by steel collars. A chromel - alumel thermocouple is introduced into the space between the furnace and the iron pipe, readings being taken on a millivoltmeter calibrated against a pyrometer. The furnace is operated at 80 v. with a V.5 Variac transformer.

No special precautions are necessary for compounds not containing nitrogen, but with the longer filling incorporating a platinum gauze, combustion must be carefully done. A semi-micro sample can be analysed as quickly as a micro-sample by Niederl's method, and the deviations from the theoretical values for a large number of samples has been ±0·1 per cent. M. E. DALZIEL

Determination of Benzoyl Peroxide in Organic Media. S. Siggia (*Anal. Chem.*, 1947, **19**, 872–873)—In iodimetric methods for peroxides the use of any common solvent other than ethyl and *iso*propyl alcohols is not recommended. Methods in which iodine is liberated are also inapplicable in presence of unsaturated compounds, as in polymerisation work. The method described uses arsenious oxide to reduce the peroxide and determines 0·01 g. of active oxygen with an accuracy to within ±0·5 per cent.

Procedure—Prepare a 0·1 *N* arsenious oxide solution containing 25 g. of sodium hydrogen carbonate per litre, and transfer 25 ml. to a 125-ml. Erlenmeyer flask. Add a quantity of the peroxide solution containing 0·005 to 0·010 g. of active oxygen. Add ethyl alcohol, if necessary, to make the solution homogeneous. If the substance for examination is a polymer, dissolve it in benzene. When benzene is used with a polymer add alcohol as usual. A precipitate of polymer may be ignored when the benzoyl peroxide content is less than 5 per cent. When the peroxide content exceeds 5 per cent. pour off the liquid from the precipitate, redissolve, reprecipitate with alcohol, and combine the extracts. Add boiling-chips to the solution and boil down to about 25 ml. in an air current. Dilute with water to 40 ml. Repeat the evaporation and dilution until all volatile organic substances are eliminated. Make just acid with *N* sulphuric acid and add 0·5 g. of sodium hydrogen carbonate. Chill the solution and titrate the excess of arsenious oxide with 0·05 *N* or 0·1 *N* iodine.

W. C. JOHNSON

Spectrophotometric Determination of Vanillin and Related Compounds. H. W. Lemon (*Anal. Chem.*, 1947, **19**, 846–849)—When solutions of *p*-hydroxyaldehydes and *p*-hydroxyketones in ethyl alcohol are made alkaline the long-wave bands of their ultra-violet absorption spectra are displaced into the high ultra-violet and their absorption intensities are considerably increased. The concentration of any one of these compounds can be determined by measuring the optical density of an alkaline solution in comparison with that of a non-alkaline solution at the wavelength of maximum absorption. The alkali concentration should be equivalent to more than 1 ml. of 0·2 per cent. potassium hydroxide solution in 100 ml., since below this figure the absorption is dependent on the alkali concentration. For *p*-hydroxybenzaldehyde, vanillin, syringaldehyde, and acetovanillone no change in absorption characteristics occurs within 22 hr., or as a result of altering the width of the slit. A procedure is also given for the analysis of mixtures of two components.

Determination of vanillin—The quantity of vanillin in the aliquot should be such that the optical density reading is between 0·2 and 1·2. Pipette two such aliquots into 100-ml. flasks, to one add 7 ml. of 0·2 per cent. alcoholic potassium hydroxide solution, and dilute each to 100 ml. with 95 per cent. ethyl alcohol that has been boiled under reflux with zinc dust and potassium hydroxide and distilled in glass apparatus. Determine the absorption of the alkaline solution in comparison with the non-alkaline solution using the Beckman spectrophotometer, after confirming the exact position of the maximum (about 353 mμ.). Calculate the concentration of vanillin from the equation $C = D/E^{1\%}_{1\,cm.}L$, where C = concentration in g. per 100 ml., D = density reading, $E^{1\%}_{1\,cm.} = 1950$ = the extinction value at 353 mμ. of vanillin in alkaline solution as compared with a non-alkaline solution, L = thickness of solution in cm.

Analysis of solutions containing two components—Any two-component mixture of *p*-hydroxyaldehydes or *p*-hydroxyketones can be analysed by the method if sufficient spectroscopic differences exist. The total concentration is obtained by determining the absorption at a coincident point on their $E^{1\%}_{1\,cm.}$ curves, and using the equation $C = D/EL$. Then the concentration of one component can be found, after determining the absorption at a non-coincident point, by the use of the equation

$$C_2 = \frac{(D/L) - E_1C}{E_2 - E_1}$$

In these equations, C = total concentration in g. per 100 ml., C_2 = concentration of component 2 in g. per 100 ml., D = optical density, L = thickness of solution in cm., E = extinction coefficient at coincident wavelength, E_1 and E_2 = extinction coefficients of components 1 and 2 at non-coincident wavelength selected. The procedure is applied, with satisfactory results, to solutions containing known quantities of vanillin (2) and syringaldehyde (1). For these two substances the coincident point is at 364 mμ. and E = 1480. A suitable non-coincident point is at 353 mμ., where $E_2 = 1950$ and $E_1 = 1080$.

Determination of vanillin in vanilla extracts—Dilute 10 ml. of the extract with absolute alcohol to 100 ml. After a few minutes filter off the flocculent precipitate that forms. Dilute a portion of the filtrate 10-fold with absolute alcohol and take two suitable aliquots of the diluted liquid in two 100-ml. flasks, add alcoholic potassium hydroxide solution to one, and dilute both to 100 ml. with absolute alcohol. Determine the absorption and calculate the vanillin content as described for the determination of vanillin (*supra*).

Determination of vanillin and coumarin in vanilla extracts—The $E^{1\%}_{1\,cm.}$ curves for these two substances coincide at 315 mμ. The absorption of coumarin is negligible at 353 mμ., where the absorption by vanillin is at its maximum. When both are to be determined lead acetate treatment and ether extraction (Englis and Hanahan, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 501) is necessary. Dilute the ether extract with absolute alcohol, prepare neutral and alkaline solutions, and determine the vanillin in the usual manner. Determine the total concentration of vanillin plus coumarin by measuring the absorption at 315 mμ. of the alkaline solution in comparison with absolute alcohol.

The absorption of an alkaline solution of coumarin alters rapidly if water is present, the maximum changing from 275 mμ. ($E^{1\%}_{1\,cm.} = 750$) to 330 mμ. ($E^{1\%}_{1\,cm.} = 380$). It is possible to distinguish qualitatively between genuine and artificial extracts by observing the change in absorption at these wavelengths during 1 hr. W. C. JOHNSON

Inorganic

Rapid Micro-Method for the Photometric Determination of Potassium. P. Jordan (*Helv. Chim. Acta*, 1948, **31**, 1483–1487)—Various conditions of time and temperature are studied for the precipitation of potassium as cobaltinitrite (*cf.* Wrangell and Beutelspacher, *Z. anal. Chem.*, 1932, **90**, 401). Complete precipitation is not attained, but the adopted conditions involve only a small and reasonably constant error. The use of a concentrated solution of sodium cobaltinitrite contributes to the reduction of the error and results in the formation of a precipitate of the composition $[Co(NO_2)_6]K_2Na.2[Co(NO_2)_6]KNa_2$. The precipitate obtained by Wrangell and Beutelspacher (*loc. cit.*) was of the composition $[Co(NO_2)_6]K_2Na$. The precipitate is dissolved in alkali and the solution treated with indole, and acidified. A red-violet colour results from the formation of nitroso-indole and the corresponding light absorption is measured.

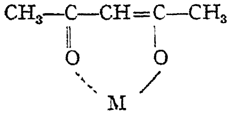
REAGENTS—*Sodium cobaltinitrite solution*—Dissolve 14 g. of the pure salt in 100 ml. of water and 4·5 ml. of glacial acetic acid. Keep the solution for a day before use, store it in a brown bottle in a refrigerator, and filter the required quantity immediately before use through a compact cotton-wool plug. *Indole solution*—Dissolve 0·15 g. of indole in 10 ml. of alcohol and dilute to 100 ml. with water. Store in a brown bottle. *Comparison solution*—Dissolve 0·40 g. of sodium nitrite in 1000 ml. of water and dilute 10-fold immediately before use. Each millilitre of the diluted solution corresponds to 0·005 mg. of potassium. This solution is used for preliminary operations, such as the choice of the colour filter.

Procedure—The sample solution may be neutral, but preferably should contain 3 per cent. of acetic acid. For the method for removing ammonium salts, see Wrangell and Beutelspacher (*loc. cit.*). Measure 1 ml. of the solution into a 10- to 15-ml. centrifuge tube with a conical bottom. Add 1 ml. of sodium cobaltinitrite solution dropwise with thorough agitation. Set aside in a vertical position for 15 to 18 hr. at 0° to 5° C., then centrifuge for 20 min. at 3000 r.p.m. Decant the clear liquid by means of a syphon made of 2-mm. tubing drawn off finely and bent at a right-angle at the inner end and equipped with a Mohr clip at the outer end; the surface of the tube is coated with paraffin wax to prevent adhesion of particles of precipitate. To the residue add 2 ml. of water without disturbing the precipitate, centrifuge for 5 min., and decant again. Repeat this operation twice. Add 5 ml. of 0·1 *N* sodium hydroxide and heat for 15 min. on a water-bath, shaking to dissolve the precipitate completely. Dilute with water and add indole solution and diluted sulphuric acid (1 + 1) in the quantities shown in the following table, the approximate quantity of potassium being found by preliminary experiment. After 5 min. determine the light absorption by the method appropriate to the instrument employed. Derive the quantities of potassium from the readings by the use of three graphs, one for each range in the table, previously determined with known quantities of potassium.

Quantity of potassium in mg.	Dilute with water to—	Add	
		indole solution	sulphuric acid
0·02–0·07	48 ml.	1 ml.	1 ml.
0·07–0·14	96 ml.	2 ml.	2 ml.
0·14–0·30	192 ml.	4 ml.	4 ml.

The scheme of dilution avoids excessive concentration at the final stage. A brownish colour is obtained beyond the concentrations employed in the method and the concentration - extinction relationship is no longer linear. The average loss of potassium is about 0·007 mg. and the dilution limit for the detection of the coloured compound is 1 part in 2,800,000. W. C. JOHNSON

Separation of Interfering Metals by Extraction with Acetylacetone in the Colorimetric Determination of Magnesium with Titan Yellow. E. Abrahamczik (*Mikrochem.*, 1947, **33**, 209–216)—In the determination of magnesium with Titan Yellow the following metals interfere: manganese, iron, copper, cadmium, zinc, bismuth, tin, aluminium, cobalt, and nickel. Separation of these metals by precipitation leads to co-precipitation of magnesium. Acetylacetone yields compounds of the type



$$CH_3{-}C({=}O{\cdots})\!-\!CH{=}C(O{-})\!-\!CH_3 \quad (\text{O, O} \to M)$$

with manganese, iron, copper, aluminium, titanium, vanadium, uranium, and certain other less common elements, and these compounds are insoluble in water but soluble in an excess of acetylacetone and in organic solvents. Thus is provided a means of removing the principal interfering elements in magnesium determination.

Procedure—To the magnesium solution containing interfering metals add sodium hydrogen carbonate to produce an alkaline reaction. Shake the solution with a solution of 1 part of acetylacetone in 4 parts of carbon tetrachloride in a cylindrical separating funnel. One or two extractions are usually sufficient to remove the bulk of the interfering metals. A film of precipitate forms on the liquid/liquid interface and remains in the aqueous phase on separation. Extract with carbon tetrachloride to remove the residual metallic compounds of acetylacetone. Remove the acetylacetone dissolved in the enolic form by acidifying slightly and extracting again. Destroy the last trace of acetylacetone by adding bromine water and reduce the excess of bromine with sulphite. Then follow the usual procedure for the determination of magnesium with Titan Yellow (*e.g.*, Kreibich and Bäumler, *Aluminium*, 1938, **20**, 528). W. C. JOHNSON

Electrolytic and Polarographic Determination of Zinc in Thorium. J. H. Patterson and C. V. Banks (*Anal. Chem.*, 1948, **20**, 897–900)—The optimum conditions for determining zinc in thorium - zinc alloys by electrolytic and polarographic methods are described. The electrolytic method can be used for samples containing from 1 to 100 per cent. of zinc; alloys with smaller zinc contents can be examined polarographically.

Procedure for the electrolytic method—Dissolve a sample of alloy containing 15 to 200 mg. of zinc and not more than 3 g. of thorium by cautiously adding 15 ml. of concentrated hydrochloric acid. After the vigorous reaction has subsided, add 5 ml. of 70 per cent. perchloric acid solution and evaporate until the perchloric acid fumes. Wash the sides of the beaker and again evaporate to fuming. Add 50 ml. of water, cool, and add 25 ml. of a 60 per cent. solution of sodium citrate. After making the solution just alkaline to methyl red indicator with sodium hydroxide solution, warm to dissolve any precipitate, dilute to 100 ml., and add 15 ml. of acetone. Electrolyse the solution with a copper-plated platinum cathode, at a current of 1 amp. for 1 hr. Wash the electrode with water with the current on and then wash with alcohol and ether, dry for 1 min. at 110° C., and weigh.

Under these conditions a suitable deposit of zinc free from any thorium, is formed.

Procedure for the polarographic method—Add carefully 50 ml. of concentrated hydrochloric acid to about 10 g. of alloy covered with water in a 600-ml. beaker. Add about 1 mg. of sodium silicofluoride and boil until the black residue is dissolved. Evaporate the solution to 20 ml., add 25 ml. of 2·0 *M* sulphosalicylic acid, and 4 ml. of 0·5 per cent. gelatin solution, followed by 20 ml. of 15 *N* aqueous ammonia. Stir until the precipitate has redissolved, adjust to pH 8·5 ± 0·2, and dilute to 100 ml. in a volumetric flask. Transfer a sample of this solution to a polarographic cell and, after removing dissolved oxygen, record a polarogram.

Add a known amount of pure zinc to a similar sample of the alloy, and treat it in the same way. The zinc content of the alloy is determined from the heights of the steps formed in the two cases.

The zinc contents of alloys containing 20 to 150 p.p.m. of zinc have been determined. Under these conditions no interference from thorium is encountered.

J. G. Waller

Polarographic Determination of Iron. L. Meites (*Anal. Chem.*, 1948, **20**, 895–897)—The polarographic oxidation of ferrous ions in an acidic buffer solution containing oxalate ions is suitable for analytical purposes. Two methods are described for preparing a solution of the sample, free from interfering ions.

Procedure 1—Dissolve a portion of the sample, containing not less than 10 mg. of iron, in 10 to 20 ml. of concentrated hydrochloric acid in a Kjeldahl flask, adding sufficient diluted nitric acid (1 + 1) to give a clear solution. Add 40 ml. of water and 20 g. of silver metal and boil the mixture vigorously for 5 min. (The silver should be prepared by adding copper to silver nitrate solution or by an electrolytic method.) Continue to boil for a few minutes after 5 g. more of silver have been added and then filter the solution into a 100-ml. volumetric flask. Add 1 g. of anhydrous sodium sulphite and dilute the filtrate and washings to 100 ml.

Procedure 2—Dissolve the sample in hydrochloric and nitric acids in the same way as Procedure 1. Evaporate the solution almost to dryness and add 20 ml. of concentrated hydrochloric acid. Again evaporate almost to dryness and then dissolve the residue in 25 ml. of 7·75 *M* hydrochloric acid, transfer the solution to a 100-ml. separating funnel, and extract it with three 30 to 40-ml. portions of peroxide-free *iso*propyl ether. Extract the combined ether solutions with 10 ml. of water, adding a saturated sodium carbonate solution until evolution of carbon dioxide ceases and the hydrated ferric oxide in the aqueous layer coagulates. If the ether layer is not colourless, re-extract it with further quantities of dilute sodium carbonate. Transfer the combined aqueous layers to a 100-ml. volumetric flask, add just sufficient concentrated hydrochloric acid to dissolve the precipitate and, after adding 1 g. of sodium sulphite, dilute to 100 ml.

An aliquot portion of the solution obtained by Procedure 1 or 2 is added to a polarographic cell containing a known volume of air-free, 1·0 *M* potassium oxalate adjusted to pH 4·8 by adding dilute sulphuric acid. The height of the anodic wave at −0·05 v. *versus* the saturated calomel electrode is proportional to the ferrous ion concentration.

The iron contents of a series of standard alloys have been determined by these procedures with an accuracy to within ±1 per cent. of the correct result.

J. G. Waller

Presence and Determination of Molybdenum and Rare Earths in Phosphate Rock. W. O. Robinson (*Soil Science*, 1948, **66**, 317–322)—The determination of 1 to 200 p.p.m. of molybdenum in various phosphate rocks by the colorimetric thiocyanate method is described. The small quantity of silica present must first be removed to avoid emulsification on extraction with *iso*propyl ether. Rhenium, if present, would give the same colour. The rare-earth content is determined on the water-insoluble residue from the sodium carbonate fusion, Reynosos' method being used to separate the calcium and phosphorus.

Reagents—*Standard ammonium molybdate solution*—Dissolve 1·149 g. of ammonium molybdate in 1 litre of water and stabilise the solution with 2 to 3 ml. of concentrated aqueous ammonia; determine the molybdenum as lead molybdate and dilute to give a solution containing 0·05 mg. of molybdenum trioxide per millilitre. *Stannous chloride solution*—Ten per cent. in diluted hydrochloric acid (1 + 9).

Procedure—*Molybdenum determination*—Mix 5 g. of finely ground rock with 15 g. of sodium carbonate in a large platinum crucible. Heat over a burner of the Meker type, at first uncovered to oxidise organic matter and then covered, to about 1050° C. and swirl every 5 min. until the mass is quiescent (about 20 min.). Cool rapidly on the sides of the crucible and detach the cake into a 250-ml. beaker half filled with water. Heat on a steam-bath for 2 hr.; break up the cake as it softens and grind to a fine slurry. Heat for 15 min. more and then filter into a large platinum dish. Acidify the filtrate with a moderate excess of hydrochloric acid, evaporate to dryness, and treat the residue with 7·5 ml. of concentrated hydrochloric acid and water. Filter off the silica and wash until the volume of the filtrate is 120 ml. Transfer to a 200-ml. separating funnel, add 5 ml. of 5 per cent. potassium thiocyanate solution, shake, add 5 ml. of stannous chloride solution, and shake again. After 30 sec. add 10 ml. of *iso*propyl ether and shake for 30 sec. Allow to stand until the ether layer is clear and transfer it to a 25-ml. measuring flask. Repeat the extraction with a further 10 ml. of ether. Dilute the contents of the flask to volume and compare with a standard prepared as follows. Measure 125 ml. of 5 per cent. sodium carbonate solution into a 200-ml. separating funnel and add 0·1 to 10 ml. of the standard molybdate solution. Add 25 ml. of concentrated hydrochloric acid and cool with swirling. Develop the colour as above and compare immediately in a colorimeter. Satisfactory comparisons can also be made in the separating funnels in the presence of the aqueous layers. *Determination of rare-earth content*—Combine the leached residues from two molybdenum determinations, dissolve in nitric acid, and evaporate. Filter off the silica, wash, and decompose it with hydrofluoric and nitric acids; add the residual solution to the main filtrate. Add 10 g. of mossy tin and heat on the steam-bath for 2 hr. Filter and determine the rare-earth metals in the filtrate by two precipitations with ammonia, two with oxalic acid in very small volumes, and an intervening precipitation with ammonia.

C. F. Herbert

Volumetric Micro-determination of Water in Minerals. C. L. Rulfs (*Mikrochem.*, 1948, **33**, 338–343)—The method is shorter and simpler than the modification of the Brush and Penfield technique developed by Hybbinette and Benedetti-Pichler (*Ibid.*, 1942, **30**, 23) and uses the Karl Fischer reagent, the end-point being determined electrometrically. Ignition of the sample is retained so that the "total" water content is still determined.

Method—Apparatus—The sample is ignited in a Pregl micro-muffle, air being circulated over the sample, placed as near as possible to the exit of the ignition tube, *T*, by directing a burner against the short leg, *L*. The collecting tube, *A*, is made of 12- to 14-mm. internal diameter resistance glass tubing, which slides loosely over the end of the ignition tube. The constriction, *b*, is about 6 mm. wide. The open end of *A* is flared and fire-polished, and the opposite end is sealed to 5 mm. of 6- to 8-mm. outer diameter tubing, *a*. During operation a blast of air is directed at *b* to ensure quantitative condensation at this point. Koch micro-burettes are used for dispensing the ethanol - water solution and the Karl Fischer reagent, and their tips should reach the bottom of the titration cell. The titration cell, as shown, is made of 30- to 36-mm. outer diameter resistance glass and is about 90 to 100 mm. high, being topped by a ground-glass cap with four outlets. Two of these carry 26-gauge platinum wire electrodes extending to 6 to 10 mm. from the bottom of the cell, the third outlet carries the burette tip, held in a rubber stopper, and the fourth is for the escape of air as the titrant is added and consists of a drawn-out capillary. Magnetic stirring avoids the need for a stirrer shaft.

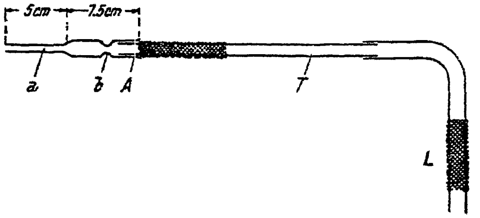


Fig. 1. *Pregl* muffle with water collecting tube

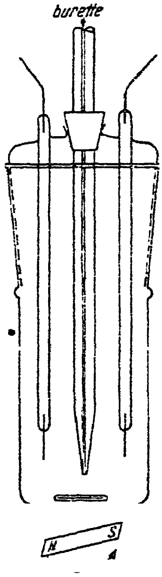


Fig. 2. Titration cell

Reagents—The solutions required are Karl Fischer reagent, a 0·2 to 0·3 per cent. solution of water in anhydrous synthetic methanol for back-titration, and a standard water-ethanol mixture. Standardise the Karl Fischer reagent by adding a weighed amount of the standard water - ethanol mixture to a measured excess of the reagent, and titrate back with the methanol solution; this gives a sharper end-point than direct taxation.

Procedure—When not in use store the dry apparatus over a good desiccant. Weigh a sample containing between 2 and 15 mg. of water into a micro-combustion boat and place it in the muffle tube, through which a gentle air stream is already passing. Slip *A* over the exit, and direct a blast of cold air against the constriction before bringing the second burner into place under the sample. When the sample reaches red heat, remove the collecting tube, and insert its narrow end, *a*, through the central opening of the titration vessel. Add 5 ml. of Karl Fischer reagent to the cell through the collecting tube and, while withdrawing it, rinse the tube with a few drops of a strictly anhydrous solvent (pyridine, methanol, ethanol, or ether), and place the burette tip immediately in position. Begin stirring, and titrate as in the standardisation; the end-point is somewhat sluggish.

Results—With samples containing not more than 13·5 mg. of water the precision of duplicate determinations is about 0·5 per cent., and the accuracy is to within ±1 per cent. M. E. Dalziel

Titanium. Polarographic Determination in Clays and Clay Products. D. F. Adams (*Anal. Chem.*, 1948, **20**, 891–895)—A polarographic method is described for determining titanium in clay, calcined alumina, leach residues, and leach liquors. The best results are obtained when the supporting electrolyte consists of 1·0 *N* sulphuric acid saturated with sodium oxalate and containing 0·8 per cent. of urea. The titanium concentration should be between $0{\cdot}06 \times 10^{-3}$ and $0{\cdot}10 \times 10^{-3}\,M$ and the ratio of iron to titanium less than 10 : 1. An empirical correction for the concentration of iron must be applied to the results.

Procedure for leach residues, original clay, and calcined alumina—Weigh a sample of dry, ground material, containing approximately 7 mg. of titanium, into a 200-ml. beaker and fuse the sample with 3·0 g. of potassium hydrogen sulphate. Cool the melt, leach it with dilute sulphuric acid, and dilute to 100 ml. in a volumetric flask. Transfer 10 ml. of the solution to a 25-ml. volumetric flask, add 8 ml. of a 25 per cent. solution of urea and sufficient 10·0 *N* sulphuric acid to give a final concentration that is 1·0 *N* with respect to sulphuric acid. Dilute to 25 ml., transfer a portion of the solution to a polarographic cell, saturate the solution with sodium oxalate and, after removing dissolved-oxygen, record a polarogram from 0 to −0·8 v. *versus* the saturated calomel electrode.

Procedure for leach liquors—Add 10 ml. of liquor, containing approximately 0·3 mg. of titanium, to 25 ml. of 1·0 *N* sulphuric acid and evaporate the solution to remove sulphur dioxide. Cool, dilute to 50 ml. in a volumetric flask, and then transfer a 10-ml. portion to a 25-ml. flask containing 8 ml. of a 25 per cent. solution of urea. The procedure is then the same as that described above.

J. G. WALLER

Determination of Chloride in Mixtures Containing Hypochlorite, Chlorite, and Chlorate. C. Loeb, J. Chabert, A. Banderet, and J. Meybeck (*Analyt. Chim. Acta*, 1948, **2**, 316–320)—In the determination of chloride with silver nitrate the various ions considered interfere in the following ways. Hypochlorite yields part of its chlorine as silver chloride while the rest passes to a higher state of oxidation. Chlorite causes low results for chloride in potentiometric determinations, but only in acid solutions. Chlorate interferes only in strongly acid solutions.

Hypochlorite is reduced quantitatively by arsenite solution and none of the oxyacid ions is reduced if the solution is alkaline. The hypochlorite is first determined, therefore, by addition of arsenite in excess, followed by bromometric titration of the excess of arsenite. Another portion of the solution is treated with an excess of arsenite, and the chloride produced by reduction plus the chloride originally present is titrated with silver nitrate in the solution buffered at pH 7·4. At this pH neither chlorite nor chlorate interferes and arsenite and arsenate are precipitated as their silver salts only after all the chloride has been precipitated.

Procedure—Run 10 ml. of the solution into an excess of 0·01 *N* sodium arsenite. Add 20 ml. of 0·1 *N* sodium borate and 4 drops of a 0·1 per cent. solution of a mixture of equal parts of phenol red and bromothymol blue. Allow to stand for a few minutes. Add 0·1 *N* boric acid until the indicator shows a pale violet colour (pH 7·4) and titrate potentiometrically with 0·1 *N* silver nitrate from a micro-burette.

From the figure found for total chloride subtract the figure already found for chlorine as hypochlorite.

W. C. JOHNSON

Rarer Elements in Qualitative Analysis. II: Uranium. T. P. Chao and S. C. Chen (*J. Chem. Educ.*, 1948, **25**, 686–687)—In qualitative analysis, uranium is detected in group III.

Procedure—After expelling hydrogen sulphide from the filtrate from group II, add solid ammonium chloride and aqueous ammonia solution until the solution is alkaline, and without filtering, saturate with hydrogen sulphide. Cold, *N* hydrochloric acid extracts all but cobalt and nickel sulphides from the precipitate. Treat the extract with aqueous ammonia solution and hydrogen sulphide, and filter the suspension. Reject the filtrate, and extract the precipitate with cold, *N* hydrochloric acid. Treat this extract with sodium hydroxide, sodium peroxide, and sodium carbonate to obtain a precipitate containing the hydrated oxides of manganese and iron, traces of the hydrated oxides of zinc, cobalt, and nickel, and if phosphate or oxalate is present, the carbonates of barium, strontium, calcium, and magnesium, and a solution containing aluminate, chromate, zincate, and peruranate. Acidify the solution with hydrochloric acid, and add a slight excess of aqueous ammonia to precipitate aluminium hydroxide and ammonium diuranate, which are filtered from the zincate and chromate solution. Dissolve the precipitate in dilute nitric acid, and add solid sodium hydrogen carbonate and a slight excess of aqueous ammonia, thus precipitating aluminium hydroxide and forming a soluble complex uranyl carbonate. Acidify the filtered solution with acetic acid and add a slight excess of sodium phosphate to precipitate white ammonium uranyl phosphate. Confirm uranium by dissolving the precipitate in dilute hydrochloric acid, concentrating the solution to a small volume, and adding sodium chloride and potassium ferrocyanide, whereupon a brown-red precipitate of uranyl ferrocyanide, which turns yellow on addition of potassium hydroxide to the suspension, should be formed.

M. E. DALZIEL

Phenoxthin as a Reagent for the Detection of Small Amounts of Palladium. O. Konig and W. R. Crowell (*Mikrochem.*, 1948, **33**, 298–299)—Phenoxthin (phenoxathin) is a sensitive reagent for palladium, and is easily prepared (*Organic Syntheses*, Vol. 2, p. 485). On the drop-reaction plate, a 10 per cent. solution of the reagent in acetone with a drop of the palladium solution in 0·1 *N* hydrochloric acid gives a yellow colour; on paper impregnated with the reagent, a yellow to red-brown spot is obtained. The reaction is sensitive to 0·01 µg. of palladium and the limiting concentration is 1 part in 100,000.

Gold salts react to give a red colour on the drop-reaction plate and a diffuse violet spot on paper; ammonia, ammonium salts, tin,^{II} cyanide, thiocyanate, fluoride, oxalate, and tetraborate also interfere. Osmate, osmium,^{III} ruthenium,^{III} and ruthenichloride ions interfere because of their colour. Mercury^{I} reduces the sensitivity of the test. Dimethylglyoxime, lead, silver, ferrous and ferric iron, tin,^{IV} cobalt, nickel, copper,^{II} nitrite, sulphate, chloride, bromide, mercuric chloride, or iridic chloride do not interfere. One part of palladium can be detected in the presence of 200 parts of platinum or 100 parts of rhodium.

The palladium and rhodium compounds can be isolated, but not those of platinum, iridium, or gold. The 2-sulphonic acid and 2 : 8-disulphonic acid derivatives of the reagent each give yellow colours with palladium, but they are less sensitive, the limit of identification being 0·1 µg., and their use has the sole advantage that gold does not interfere.

M. E. DALZIEL

Thio-Acids as Drop Reagents for Palladium. O. Konig and W. R. Crowell (*Mikrochem.*, 1948, **33**, 300–302)—Aqueous solutions of thioglycollic and thiomalic acids give yellow colorations with bivalent and quadrivalent palladium salt solutions at pH values between 0·5 and 9·0; the limit of identification is 0·05 μg. of palladium and the limiting concentration 1 part in 100,000. At concentrations greater than 5 parts per 1000, a yellow precipitate forms; this dissolves readily on dilution.

At concentrations exceeding 10 g. per litre, gold, platinum, and iridium salts give white, amorphous precipitates, which dissolve on dilution to give colourless solutions; rhodium salts give a pink precipitate, which dissolves on dilution to give a pink solution and turns brown on standing, or yellow if the solution is very dilute. Brown iridic chloride is reduced to a colourless tervalent form.

One part of palladium can be detected in presence of 250 parts of gold, platinum, or iridium, or any mixture of these and in presence of 25 parts of rhodium with or without 75 parts of a mixture of the other three. Ruthenium gives a grey spot, osmium a greenish-grey spot, mercuric mercury a yellow precipitate, silver an orange precipitate, and cupric copper a yellow precipitate. Ferric iron reacts only in the presence of ammonia, a violet coloration being formed.

The following do not interfere: nitrate, sulphate, thiocyanate, chloride, bromide, iodide, fluoride, chlorate, perchlorate, oxalate, tetraborate, ammonia, ammonium, and dimethylglyoxime. Cyanide inhibits the reaction and nitrite gives a deep violet spot, but 0·1 μg. of palladium can be detected in the presence of 10 μg. of nitrite.

The compounds formed cannot be isolated, but the composition of the palladium thioglycollic acid compound has been deduced by indirect methods.

M. E. Dalziel

Electro-reduction of Periodate at the Dropping Mercury Electrode. Part I. Behaviour in Acidic Solutions of pH less than 3. R. H. Coe and L. B. Rogers (*J. Amer. Chem. Soc.*, 1948, **70**, 3276–3281)—In the pH range from 0·8 to 3·0, periodate ions give two reduction steps, which represent reduction to iodate and to iodide. These steps have a limited application for analytical purposes. Acidic solutions of potassium metaperiodate can be determined with an accuracy to within ±1 per cent. by using the second step, but solutions of orthoperiodic acid cannot be determined with an accuracy better than to within ±2·5 per cent.

J. G. Waller

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 986)—The crystallography of ascorbic acid is described under the headings given in the first paper of the series (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579).

M. E. Dalziel

Polarography. Some Factors Affecting Drop-Time. F. L. English (*Anal. Chem.*, 1948, **20**, 889–891)—The effect of a number of factors on the drop-time has been examined. These factors include vibration, the applied voltage, the purity of the mercury, the cleanliness of the capillary tip, the current flowing, and the nature of the solution.

Slight vibration of the capillary has a measurable effect on the drop-time. The purity of the mercury has little effect, ordinary laboratory-grade material giving the same drop-time as carefully purified material. Liquid amalgams of various metals, however, have a considerable effect on the drops. Traces of oil on the capillary tip produce irregular drops, but this can be avoided by immersing the tip in ethyl alcohol when not in use.

The presence of capillary-active substances in the solution has a considerable effect as does the solvent used, but the effect of the concentration of the supporting electrolyte is small.

The results show that for accurate calculations involving the Ilkovic equation, it is important to determine the drop-time under the exact conditions of the analyses.

J. G. Waller

Reviews

PHOTO-ELECTRIC METHODS IN CLINICAL BIOCHEMISTRY. By G. E. DELORY, M.Sc., Ph.D. Pp x + 90. London: Hilger & Watts, Ltd. 1949. Price 15s.

There is no doubt that photo-electric methods of analysis are popular. From their introduction somewhere about twenty years ago they have made rapid strides in popularity, and these strides became almost those of seven-leagued boots with the appearance on the market of suitable photo-electric instruments. It is a truism that any method of analysis that lends itself to the quantitative application of visual colorimetry can be better applied photo-electrically; for the uncertainties and vagaries of the human eye are nowhere more apparent than in the laboratory.

With so wide a range of application, photo-electric analysis could clearly not wait long before its own literature came into existence. Much of the pioneer work in the adaptation of suitable methods was carried out on Messrs. Hilger's instruments, and as their instruments become more and more widely distributed the makers assist the users by the publication of books such as the one under review.

It describes modifications of a number of well-known biochemical colorimetric determinations suitable for use with Hilger instruments, the range of tests described covering all those more commonly wanted. Needless to say, only slight further modification would be required to adapt the tests as here recorded for use in any other photo-electric instrument. In addition, three introductory chapters describe simply and adequately the general principles of colorimetry, of colorimetric measurements and of the special technique of photo-electric colorimetry. These chapters are followed by descriptions of the Hilger instruments.

The subject is set out in very simple language, and the author has adopted very straightforward means for describing the various methods of test. If some of these, at a casual glance, seem to differ in not unimportant details from earlier descriptions of the same methods appearing elsewhere, no doubt the author is sure of his ground and can recommend the latest version.

K. A. Williams

RECENT ADVANCES IN ANALYTICAL CHEMISTRY. FRONTIERS IN CHEMISTRY. Volume VII. Edited by R. E. BURK and OLIVER GRUMMITT. Pp. 209. New York and London. Interscience Publishers. 1949. Price 27s.

This volume is the seventh of the Frontiers in Chemistry series published under the auspices of the Western Reserve University, Ohio. As with the previous volumes, this book gives permanent form to a number of lectures on related subjects given at the University by a group of specialists.

Those unfamiliar with the series should be warned that these volumes do not give as exhaustive a treatment of the subject as their titles may appear to suggest. For example, if the present title were prefaced with the word "Some," this would in no way detract from the merits of the book and would be generally fairer to the authors and the reader.

The contents include: Voltammetry (Polarography) and Amperometric Titrations, by I. M. Kolthoff; Inorganic Analysis with Organic Reagents; Some Recent Colorimetric and Gravimetric Organic Reagents, both by J. H. Yoe; Applications of Infra-red Spectroscopy in Analysis, by O. Beeck; Electron Microscopy and Micro-analysis, by J. Hillier; Fractionation, Analysis and Purification of Hydrocarbons, by F. D. Rossini; Applications of the Mass Spectrometer, by J. A. Hipple.

Electron Microscopy and Infra-red Spectroscopy have already been dealt with in Volume IV of this series, "Major Instruments of Science and their Application to Chemistry" (reviewed in *The Analyst*, 1946, **71**, 345), but the treatment in the present volume is not only by a new team of specialists, but also takes account of three years' progress in these rapidly advancing subjects; duplication is thus more apparent than real. Both these and several of the other subjects included have received attention at meetings of the Physical Methods Group of our Society; these more detailed accounts of recent progress will be welcomed by those whose interest has already been stimulated in such branches as Polarography and Mass Spectrometry.

Reading these essays emphasises once again how diverse have become the tools and methods of modern analytical chemistry or rather chemical analysis. The only feature remaining in common appears to be the ultimate purpose of the operation. This very diversity precludes authoritative comment on all the sections of the book. Those within the reviewer's own field of interest are authoritative, lucidly written and stimulating to read; those dealing with less familiar matters appear to be of an equally high standard.

No analyst can fail to profit by attending this course of lectures through the medium of the printed word; teachers of chemistry should find this account of modern advances particularly valuable. Potential research workers in the field are specially recommended to read Professor Yoe's "Outline for Development of a New Colorimetric Method."

B. S. COOPER

ALCOHOL: A FUEL FOR INTERNAL COMBUSTION ENGINES. By S. J. W. PLEETH, B.Sc., F.Inst.Pet. Pp. xv + 259. London: Chapman and Hall Ltd. 1949. Price 28s. net.

Modern civilisation runs on fuel, and a constantly recurring exercise of economists is to estimate how long it will be before the natural resources of fuel are exhausted. The estimates are very variable and agree only that an end will come. However remote that may be, the deduction is general that natural assets must be conserved and other sources of energy must supplement if not supersede them. For the internal combustion engine, as we know it, liquid fuels will probably always be essential and any that can be produced in unlimited quantity must be of value. Ethyl alcohol is not an ideal fuel but it is an important one having many desirable characteristics, including that of production from vegetable matter available throughout the world and renewable annually.

The author first surveys solid and liquid fuels and then describes the production of ethyl and other alcohols from natural raw materials and by synthesis. Modern methods of dehydration are indicated by flow-sheet diagrams. The principles of the internal combustion engine are dealt with and the essential requirements and characteristics of liquid fuels are discussed, including fuel volatility, knock rating, water tolerance, gum formation and subsidiary effects such as corrosion and cylinder wear. About 16 pages are devoted to the testing of alcohol and alcohol blends.

The methods of test described are adequate for the purpose, but no mention is made of the Karl Fischer reagent when the water content is required with accuracy.

The composition of Power Methylated Spirit given is not quite in accordance with the regulations, and the fact that it must be coloured with a specified amount of dye is not referred to.

The Appendix gives 12 tables relating the specific gravity or density of alcoholic solutions with their strength. The first six tables, dealing with aqueous ethanol, are labelled "by permission of the U.S. Bureau of Standards" and the seventh is an abstract from the British Official Tables. It is pointed out that there is a discrepancy between Table VII and the previous ones and inspection shows that the differences are in the higher strengths and are of the order of 0·1 per cent. alcohol. It may, however, be mentioned that the first six tables do not agree with those given in the United States Pharmacopoeia or in the Methods of Analysis of the Association of Official Agricultural Chemists, all of which are in agreement with the British Official Tables to within 0·01 per cent. alcohol. It would appear that the American Tables cited are not the current ones and caution should be observed in their use.

Although at the moment power methylated spirit is little used in this country as a fuel, the position may change in the near future. Coal of poor quality must be processed at the collieries, and some of the products of low temperature carbonisation and of hydrogenation will need to be blended before they are suitable as motor fuels. Alcohol is particularly promising for such blending and the information given in this book will be valuable to those concerned in marketing such products capable of competing with petrol.

J. R. NICHOLLS

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 0d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

The Reichert-Polenske-Kirschner Process. (Test for Butter Fat.) *To be reprinted.*

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *To be reprinted.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajaput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 1. Determination of Arsenic. *Out of print.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held on Wednesday, October 5th, 1949, at 6.45 p.m., in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The President, Mr. George Taylor, O.B.E., F.R.I.C., was in the chair. The following papers were presented and discussed: "The Calculation of the Botanical Composition of Wheat Flours and Offals from the Chemical Analysis," by J. Straub, Chem.Ing.; "A Photo-electric Method of Determining the Colour of Flour as Affected by Grade, by Measurements of Reflecting Power," by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., and W. Martin, B.Sc.; "Experiments in the Photo-electric Recording of Flour Grade by Measurements of Reflecting Power," by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., A. J. Amos, B.Sc., Ph.D., F.R.I.C., and W. Martin, B.Sc.

NEW MEMBERS

Manuel Carlos Alvarez Querol, D.Sc. (Madrid); Eric Morrison Bavin, B.Sc. (Lond.), F.R.I.C.; Parmatma Sarup Bhatnagar, M.Sc. (Punjab); John Herbert Briston, B.Sc. (Lond.); Matthew Clasper, A.R.I.C.; Amarendra Nath Das, B.Sc.; George Arthur Frederick Harrison, B.A. (T.C.D.), A.R.I.C.; John Francis Herringshaw, B.Sc., Ph.D. (Lond.), A.R.C.S., D.I.C., A.R.I.C.; Francis Horricks, A.R.T.C. (Salford); Hugh Cardozo Hornsey; Ronald Alfred Jamsett, B.Sc. (Lond.), A.R.I.C.; George Frederick Somers, B.Sc., Ph.D. (Lond.), Ph.C.; Geoffrey Arthur Wood, B.Sc. (Lond.), A.R.C.S., A.R.I.C.

DEATHS

We regret to have to record the deaths of

Sir Robert Howson Pickard (Honorary Member).
William Pearson Skertchly.

The Assay of Curare and Curarimimetic Substances

The following four papers were read at a meeting of the Biological Methods Group on Thursday, October 21st, 1948.

The Assay of *d*-Tubocurarine Chloride on the Isolated Rat-Diaphragm

A Statistical Examination

By G. A. MOGEY, J. W. TREVAN and P. A. YOUNG

The use of the Bülbring rat diaphragm preparation (Bülbring[1]) for assay of curarising drugs was described by one of us (J. W. T.) at the meeting of the Pharmacological Society in January, 1946, and Chou[2] has independently described a similar method. We have now examined the method statistically.

The preparation was essentially the same as that described by Bülbring except for the electrode for stimulating the nerve. This is represented in Fig. 1. It consists of a $\frac{1}{4}$-inch glass tube, A, with an oblique opening at the bottom over which is stretched a rubber membrane, B, with a hole in the middle made by boring slowly with a wet darning needle; pushing the needle straight through will split the rubber; silk thread is attached to the central

Linlithgow Library.

end of the phrenic nerve, C, which is gently drawn through the hole in the membrane. One silver - silver chloride electrode, E1, is placed inside the tube, which contains a little Ringer - Locke solution, and another, E2, in the bath which contains the rat diaphragm, D. The rubber membrane dips into the bath and a potential difference between the electrodes causes stimulation of the nerve where it passes through the membrane. The current should pass down the nerve to avoid anodal block. Slightly supramaximal stimuli, varying in voltage with the size of the hole in the membrane, are used; 1·5 volts is about the maximum necessary. The stimulus is a short pulse of direct current less than about 1 m.sec. in duration. We use 0·33 m.sec. Longer stimuli produce repetitive responses (Mogey and Trevan[3]) in the nerve, with tetanic responses of the muscle, varying in height from nerve volley to nerve volley, and often during a long assay diminishing in average height too much for accurate work. Ringer - Locke solution of the following composition, with oxygen bubbled through, is used:

NaCl	90 g.
KCl	4·8 ,,
$CaCl_2$	2·4 ,,
Glucose	10·0 ,,
$NaHCO_3$	5·0 ,,

Distilled water to 10 litres.

Tyrode solution gives rather smaller regressions of response on dose.

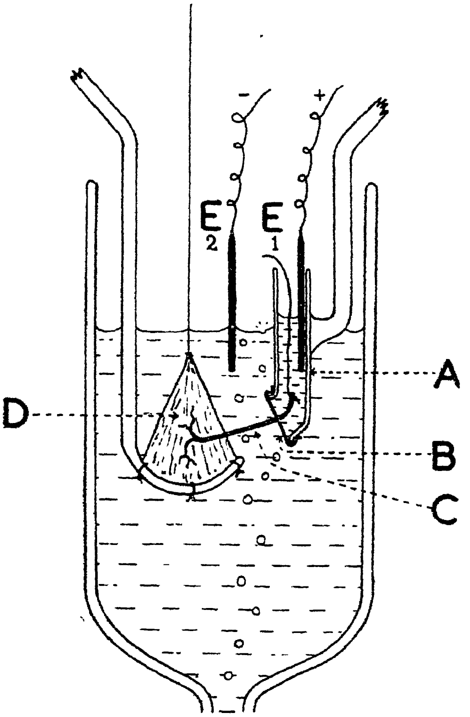

Fig. 1. Diagram of isolated organ bath showing: A, glass tube sealed at lower end with B, a thin rubber membrane through which C, the phrenic nerve passes. The diaphragm segment, D, is tied to a glass supporting rod below, the apex being connected to a writing lever by a thread. A potential applied between the silver - silver chloride electrodes E1 and E2 stimulates the nerve where it passes through B.

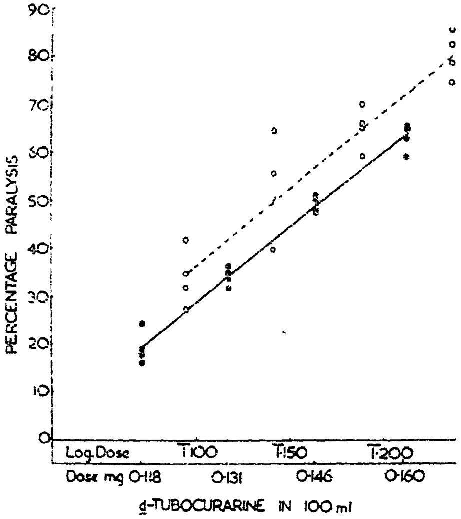

Fig. 2. Dose - response lines of rat diaphragm to *d*-tubocurarine. Solid circles: Experiment 1. Open circles: Experiment 2.

The accuracy of the method has been examined by applying the analysis of variance to a series of randomised doses. Four doses, each 0·9 of the next, were added to the bath, the Ringer - Locke solution being replaced between successive doses. The doses were given in an order determined from a randomly chosen 4 × 4 Latin square. The first two or three doses administered after the isolation of the diaphragm show a fairly rapidly increasing effect, which will be the subject of another communication. Three or four preliminary doses are therefore given before the actual assay is begun. These preliminary doses can be used to determine the approximate equivalent doses of the standard and test. The response after these preliminary doses sometimes still shows a significant alteration, but it is of smaller dimensions and rarely if ever of such extent as to invalidate the experiment by the occurrence of complete paralysis with any dose. This alteration in response can be eliminated from the calculation of the error by the variance analysis.

Fig. 2 shows two examples of the response to such a series of 4 × 4 doses of a solution of *d*-tubocurarine randomised as described, with a regression line fitted to each. Linearity of regression is found between 20 per cent. and nearly 100 per cent. paralysis in different experiments. Above about 85 per cent. the variance alters, so that for accurate error estimation, doses should be so adjusted that the paralysis is between 20 and 80 per cent. Table I shows the arrangement of the injections and the recorded responses measured on a smoked drum for experiments where A, B, C and D represent the doses in increasing magnitude from A to D, A being 0·9 of B and so on. Underneath each letter corresponding to a dose is the percentage paralysis, the contraction being measured on the kymograph to 0·25 mm. at the time when the maximum effect was attained. The doses were administered in the order represented by reading across each row successively.

TABLE I

Percentage paralysis of a rat diaphragm produced by the doses of *d*-tubocurarine A, B, C and D, in Experiment 1 (Fig. 2)

A/B = B/C = C/D = 0·9

D 64	A 25	B 35·5	C 48·25
B 34·5	D 60·0	C 51·5	A 17·75
C 48·5	B 32·25	A 16·5	D 67·0
A 18·75	C 51·75	D 67·0	B 36·5

The result of the analysis of variance is given in Table II. The rows and columns both show the effect, if any, of an alteration of response with time. The columns give a measure of the effect over the time taken for the application of four doses; the rows give the mean effect over the time taken for sixteen doses. In the two examples above, in Experiment 1 there is no significant effect of this kind; in Experiment 2 the variance of the rows is significant, but that of the columns, though large, is not significant. The calculation of the P 0·95 ratios gives, using only the error, with six degrees of freedom for one pair of

TABLE II

ANALYSIS OF VARIANCE OF THE RESULTS PLOTTED IN FIG. 2

	N	Expt. 1 Mean square	Expt. 2 Mean square	F	P
Rows	3	7·40	109·64	6·17	< 0·05
Columns	3	1·06	65·18	3·67	< 0·2
Doses	3	1506·47	1713·51		
Error	6	11·84	17·87		
	15				
Regression coefficient of % paralysis on $\log_{10}$ of dose, (*b*)		330	348		

observations, 1·085 (Experiment 1) and 1·102 (Experiment 2) and for eight pairs of observations 1·028 (Experiment 1) and 1·034 (Experiment 2). Eight pairs of observations correspond to the division of sixteen doses between test and standard solutions. This omits that part of the error that would be introduced when the means of the doses of standard and test differ. They can at all times, by means of the preliminary doses, be placed at not more than 10 per cent. apart and the appropriate correction, though easy to calculate, will generally be very small because *b* is almost invariably greater than 10 times and may be over 50 times its standard error. The value of *b* is very high. With $b = 330$, a drop of 10 per cent. in the dose produces an arithmetical difference of 15·1 per cent. in the percentage paralysis, *e.g.*, from 60 to 44·9 per cent. Since the latter usually corresponds to about 2·5 to 3 cm. in the tracing on the drum, the effect due to error of measurement of the tracing is negligible.

TABLE III

DATA EXTRACTED FROM ANALYSES OF CONSECUTIVE ROUTINE ASSAYS OF *d*-TUBOCURARINE

(1) Assay No.	(2) *b* slope	(3) V*b*	(4) V error $n = 10$	(5) V order $n = 3$	(6) Mean time per dose (min.)	(7) Ratio of P 95 limit to mean
1	375	101	6·3	83·4	9·5	1·017
2	307	646	55·1	755·0	12·7	1·064
3	209	106	13·2	255·0	11·4	1·046
4	263	220	27·2	745·0	15·1	1·052
5	291	422	36·0	169·0	12·3	1·054
6	310	510	31·9	103·0	7·7	1·057
7	339	37	4·6	81·5	5	1·016
8	323	2350	66·0	35·2	5	1·061
9	332	173	18·3	116·9	5	1·034
10	338	43	3·4	30·4	5	1·014
11	361	540	29·3	135·4	5	1·040
12	290	455	24·3	21·3	5	1·042

Table III shows a series of routine tests. In these a 4 × 4 Latin square design was also used, with two doses of standard and two of test, each pair of doses being in the same ratio one to another, usually about 1·3. The first six tests were read at equilibrium, that is to say, when the dose of tubocurarine had produced its maximum effect on the contractions, which remain almost constant even if the dose is left in for some hours. The table gives in the successive columns, (1) the identification number of the assay, (2) *b*, the coefficient of the regression of percentage paralysis on $\log_{10}$ dose, (3) V*b*, the variance of *b*, (4) V (error), obtained by pooling the error term of the analysis of variance, the variance of columns, and the component of the mean square for elimination of lack of parallelism of the regression, neither of the latter two being significantly higher than the error term. This gives 10 degrees of freedom $(6 + 3 + 1)$. Column (5), V (order), shows the variance eliminated for rows. Amongst the six tests under discussion, only No. 6 shows an order variance that is not significant. Column (6) gives, in minutes, the mean duration of action of the doses. Column (7) gives the ratio of the upper fiducial limit $(P = 0·95)$ to the mean activity of test in terms of standard.

Response after fixed time—Examples 7 to 12 in Table III show the results of the analyses of assays in which, instead of waiting for each dose to produce its maximum effect, it was allowed to act only for precisely 5 minutes; the degree of paralysis at that time was used as a measure of the effect. This is similar to the procedure used by Chou[2] except that his experiments were not designed to eliminate the change in sensitivity. He used "bracketting" of doses to estimate the relative potency of the test and standard, which renders error determination difficult and uncertain.

Examination of the original records of our preliminary four-dose experiments with one solution shows that a linear regression still results with observations at this time. In using the paralysis at a fixed time it is important to ensure that the dose is injected into the bath well away from the muscle. If the concentrated solution impinges on the muscle, the local rise in concentration will markedly accelerate the rate of onset of paralysis, although the final degree of paralysis is the same when equilibrium is established. The figures given for examples 7 to 12, obtained by the standard time interval method, show that the value of *b* is rather higher than for examples 1 to 6; the error term after elimination of order effects is nearly the same on the average (although this is greatly influenced by the high value, 66, for number 8). Whilst order mean square is still significant in 4 out of 6, it is considerably lower than that for examples 1 to 6. This is not suriprsing, as the total duration of the "fixed-time" test is less than half that of the other method, and the sensitivity of the preparation increases with time.

Experiments with Tyrode solution—A number of assays of irregular design in which Tyrode solution instead of Ringer - Locke solution was used, gave results for which the order effect could not be eliminated, and a value of b significantly less than that given in either of the Ringer - Locke series. Table IV gives the mean values for various elements of the variance analysis. This table shows that the mean square for (order + error) is not significantly different for experiments with Tyrode solution (I), and Ringer - Locke solution (II), using the equilibrium effect as index. The error of assay with Tyrode solution would be higher because b is significantly less ($t = 11, p < 0\cdot001$). The mean square for (order + error) is much less for series III. This is due entirely to reduction in the order term as is shown by the figures for mean square for error, order being eliminated. It seems likely that a proper design for Tyrode solution would eliminate as large a mean square for order, for the average interval between doses was about the same as for series II, but the disadvantage of the lower slope for the Tyrode would remain. The series of mean squares for error (order eliminated) in both cases is heterogeneous (χ^2 test) as are the slopes for series I and II. The series of slopes (b) for I and II are also heterogeneous, but not that for III. In other words, the variance of the slopes in series III is less than the (heterogeneous) mean variance for error. The slope for series III is larger than that for series II ($t = 6\cdot47, p < 0\cdot001$). On all accounts, therefore, the method of series III is to be preferred as likely to have the smaller error, and at the same time to be more rapid and convenient in execution.

TABLE IV

MEAN VALUES FROM THE ANALYSES OF THREE SERIES OF ROUTINE *d*-TUBOCURARINE ASSAYS

Series		Mean square order + error	n	Mean square error	Slope (b)	Variance of mean b
I	Tyrode irregular design ..	105·8	88	—	177	85
II	Locke equilibrium readings	102·9	78	24·3	291	34
III	Locke dose acting for 5 min. only	34·9	78	28·3	337	16·5

SUMMARY OF THE METHOD

A Bülbring preparation of the rat diaphragm and phrenic nerve is suspended in 100 ml. of oxygenated Ringer - Locke solution at 37° C. The nerve is stimulated by approximately rectangular pulses of direct current passing down the nerve, the pulses not exceeding 1 m.sec. in duration. Doses of the standard preparation are added from a "tuberculin" syringe, with three washes with Ringer - Locke solution between successive doses, until the response becomes uniform. The assay proper then starts with doses chosen so as to keep the responses between 20 and 80 per cent. paralysis; suitable concentrations can be selected by consideration of the effectiveness of the preliminary stabilising doses. Each dose is allowed to act for 5 minutes by stop clock. Three washes with Ringer - Locke solution are used between each pair of doses, taking about 5 minutes altogether. The four doses—two of standard and two of test—are assigned at random to the elements of a 4 × 4 Latin square to determine the order of testing; the analysis of variance determines whether an order effect is significant and can be eliminated.

DISCUSSION

The accuracy attainable with this method is very high for a biological assay. When the test gives fiducial limits ($P = 0\cdot95$) little more than 1 per cent. either side of the mean, as it does with considerable frequency, it is of some interest to examine the possible elements of the variance. We can identify: (1) errors of measurement of dose, (2) errors of measurement of volume of Ringer - Locke solution and (3) inherent variability of response of endplate; besides (4) possible errors due to variations in temperature, oxygenation, etc. Then when the fiducial limits are just over 1·0 per cent., the standard deviation will be 0·5 per cent., and the variance plotting the dosage - response curve, testing its linearity, and estimating its slope and the ED 50 and its fiducial limits. Typical data obtained in the series of experiments carried out during the present year are given in Table I, and illustrated in Figs. 1 and 2.

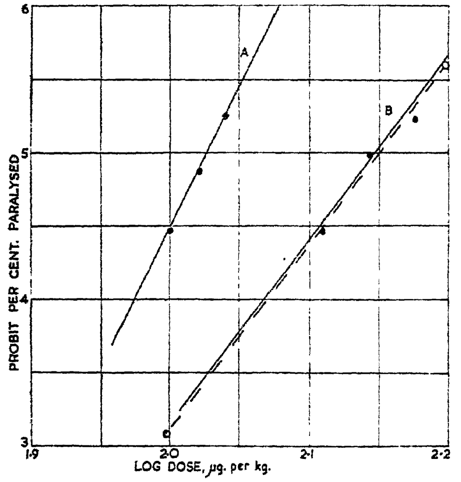

Fig. 1. Log dosage - probit paralysis lines for two specimens of *d*-tubocurarine in the mouse. The broken line is computed

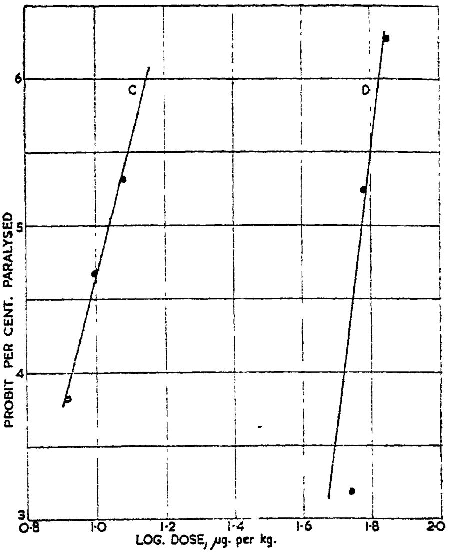

Fig. 2. Log dosage - probit paralysis lines for: (C) *d*-OO-dimethyltubocurarine and (D) *d*-tubocurarine in the rat

TABLE I

DATA UPON WHICH CURVES A, B, C AND D IN FIGS. 1 AND 2 ARE BASED

Curve	Drug	No. of animals	Dosage level μg. per kg.	No. paralysed /total No.
A	*d*-TC	60 mice	100	6/20
			105	9/20
			110	12/20
B	*d*-TC	30 mice	130	3/10
			140	5/10
			150	6/10
C	DME	24 rats	8·3	1/8
			10	3/8
			12	5/8
D	*d*-TC	22 rats	55	0/7
			60	3/5
			70	9/10

Although most of the tests supplied similarly steep estimates of slope, the standard errors attaching to these individual estimates were large, owing to the small size of the dosage groups and to the necessarily restricted ranges of doses administered. For curve A, for example (Fig. 1), the slope is 19 ± 10, so that the fiducial limits ($P = 0{\cdot}95$) of the ED 50, if it were estimated from these data alone, would be infinite.

For these particular experiments, however, reliable estimates can be obtained by using a pooled value of the slope of the log dose - probit line. The tests cover a total of 662 mice, and provide 34 separate estimates of slope. These prove to be in good agreement with one another ($\chi^2 = 24{\cdot}1$ with 33 degrees of freedom), and supply a pooled estimate of slope 16·3 ± 2·0. There were more than two dosage groups in 14 of the series of 34, and in none of these were the deviations from linearity significant.

With *d*-tubocurarine and its dimethyl ether dosage - paralysis curves may be obtained also in the rat in the way described above. A typical curve for each substance is illustrated in Fig. 2. These curves are based on the data given in Table I.

Whilst *d*-tubocurarine may be satisfactorily estimated in the mouse, for various reasons discussed below the rat is more suitable for the estimation of preparations of *d*-OO-dimethyltubocurarine. Experiments carried out during the present year with this substance in 261 rats have provided twelve separate estimates of the log dose - probit line. These also prove to be in good agreement ($\chi^2 = 15{\cdot}1$ with 11 degrees of freedom) and supply a pooled estimate of slope 14·8 ± 2·0. There were more than two dosage groups in seven of the experiments and in none of these were the deviations from linearity significant.

Examination of the figures on which the above conclusions are based indicates that although the slopes of the log dose - probit lines, both of *d*-tubocurarine in the mouse and of *d*-OO-dimethyltubocurarine in the rat, do not differ significantly from one test to another, their position may alter somewhat on different occasions. It is consequently necessary to employ the appropriate standard preparation in each test.

The effect of rate of injection—The question naturally arises whether or not it is necessary to inject the curarising substance at a constant rate. Experiments with *d*-tubocurarine in mice indicate that, within wide limits, the speed of intravenous injection makes little difference to the response to the curarising agent. A typical experiment is illustrated in Table II.

TABLE II

EFFECT OF RATE OF INTRAVENOUS INJECTION UPON THE RESPONSE TO *d*-TUBOCURARINE IN MICE

No. mice	Rate of injection in ml. per min.	Dosage levels in µg. per kg.	No. paralysed /Total No.
30	1	100	3/10
		105	4/10
		110	6/10
30	9–10	100	3/10
		105	5/10
		110	6/10

It is evident from these figures that it would be difficult to show a difference between the responses to injections at a rate of 1 ml. per minute and at a rate of 9 to 10 ml. per minute. Consequently no serious error is likely to arise if we fix a rate of injection at 1 ml. per minute.

The effect of the body weight of mice—As mice in any batch may vary slightly in body weight, we thought it important to investigate whether or not variation in the weight of mice used affected the response to *d*-tubocurarine. The results of an experiment set out in Table III indicate that it would be difficult to show a significant difference in response between mice weighing 14 to 17 g. and those weighing 20 to 26 g. We concluded therefore, from this and similar experiments, that no serious error was likely to arise owing to differences in the weights of mice in an experimental batch if the weight range was less than 5 g.

Repeated use of animals—At the dosage levels used in the experiments described above the loss of righting reflex seldom lasts long, and deaths are very infrequent. Recovery appears to be complete, for we found that the same batch of mice or rats, when used once weekly for two or more successive weeks, continued to give responses suitable for assay purposes.

The fact that mice or rats may be used repeatedly suggests that the accuracy of our assay might be increased by adopting a cross-over design. Such a design, however, might entail an unacceptable delay in the completion of the test.

Effect of strain of mice—During these tests we used on different occasions two different strains of white mice obtained from two suppliers. It was evident from a number of tests that one strain was more sensitive than the other. This fact is exemplified by comparison of Table II with Table III. The same sample of *d*-tubocurarine was used on both occasions, but a different strain of mice was employed. If a standard *d*-tubocurarine and a single strain of mice is employed in each test, no error is likely to arise as a result of strain differences.

Duration of paralysis as a basis of assay—In a previous publication[3] it was suggested that the mean duration of paralysis, as indicated by the loss of righting reflex, is a continuous variate which may be usable for the estimation of curarising activity. From the slopes of the various log dose - response lines obtained for *d*-tubocurarine and its dimethyl ether in the mouse, rat and rabbit, the response of the rat to the dimethyl ether appeared to offer the

TABLE III

EFFECT OF WEIGHT OF MICE UPON THE RESPONSE TO *d*-TUBOCURARINE

No. mice	Range of weight in g.	Dosage level µg. per kg.	No. paralysed /Total No.
40	14–17	71	5/20
		79	15/20
40	20–26	71	5/20
		79	10/20

best possibilities of use. Analysis of these and subsequent results, however, shows that the two following objections make this response unsatisfactory for assay purposes—

(1) The variance of the response at each dosage level is high.

(2) At higher dosage levels deaths of animals not infrequently occur. In practice this is wasteful, and in theory it is difficult to know how to treat deaths.

DISCUSSION

Experimental considerations—Use of the subcutaneous or intraperitoneal routes of administration of a drug introduces variations in the responses of individual animals due to (1) different rates of absorption into the blood stream from the site of injection and (2) different rates of elimination of the drug either by its excretion or destruction. If the intravenous route of administration is employed, variation due to different rates of absorption becomes negligible. Since *d*-tubocurarine takes effect, if at all, within 1 minute of its intravenous administration to the mouse, variation due to individual differences in its elimination from the body must be slight. Since we have shown[4] that the dimethyl ether is slowly eliminated by the rat, this argument must apply with even greater force to this compound in this animal. For these reasons it seems probable that the intravenous administration of curarising drugs, followed by observation of whether or not the righting reflex is lost, reduces variation between individuals to that based upon the conveyance of the drug to and the responses of the neuromuscular junctions themselves. The loss of the righting reflex is itself a mean effect, based upon the responses of a large number of neuromuscular junctions. In effect, therefore, we appear to be studying in each animal the mean response of a large number of neuromuscular junctions to the curarising agent. It may be for these reasons that the dose - response curves obtained by our method are particularly steep, the slopes being two to three times as great as those obtained by Skinner and Young or by Pick and Richards. It is worth noting that use of the duration of paralysis as a basis for assay would introduce variation due to differing rates of eliminating the drug.

When the slope of the dose - response curve is steep it is evident that the accuracy of administration of the dose becomes extremely important. It is for this reason that we have adopted the procedure of giving to the mouse 0·4 ml. per 20 g. and using dilutions of the drug made serially by a factor of 0·9 to 0·95.

The question of the choice of animal for assay of curarising activity depends partly on the relative sensitiveness of each animal to the substance concerned and partly upon the cheapness and availability of each species. It is well known that the mouse is more sensitive to *d*-tubocurarine than to its dimethyl ether. When the latter substance is to be estimated the mouse does not appear to be suitable, since the ether is derived from *d*-tubocurarine and the activity measured might be merely that of an impure sample of *d*-tubocurarine. The rat, however, is about five times as sensitive to *d*-OO-dimethyltubocurarine as it is to *d*-tubocurarine itself, and is consequently a suitable animal for assay of the ether. When *d*-tubocurarine itself is to be estimated the mouse appears to be more suitable, as it is more readily available, and more sensitive to this substance than to its derivative.

If there is any question of one substance being contaminated with the other, assay in both the mouse and the rat should be sufficient to reveal this and to indicate the approximate proportions of each in the mixture (see Chang and Gaddum[4]).

The planning of assays—The rabbit head-drop test elaborated by Dutta and MacIntosh,[5] which is alone regarded as acceptable for the biological assay of impure preparations of *d*-tubocurarine chloride,[6] provides fiducial limits ($P = 0{\cdot}95$) lying within ± 10 per cent. of the estimated activity. It can easily be calculated from the standard formula giving the fiducial limits that, if we were prepared to assume that in all future assays the slope β of the log dose - probit line would have the value 16·3 indicated by the series of tests discussed above, then, subject to one restriction, the same degree of accuracy as in the official rabbit head-drop test would be attained by using in an assay a total of 64 mice, 32 for the standard and 32 for the test preparation. The restriction is that all the doses administered should be within the dosage range corresponding to reaction percentages lying between 25 and 75 per cent. It is necessary to make a proviso of this sort because the weight nw ($= nz^2/pq$ in the usual notation) attaching to a dosage-group of n animals varies with p, the expected proportion responding; if p lies between 25 and 75 per cent., w is at least 0·54.

In fact, of course, we can never be certain that the slope β will remain constant, and it is preferable to estimate it afresh in each assay, that is, to treat each assay as self-contained. From this point of view it is impossible to predict accurately the number of mice that should be used to obtain the required degree of accuracy, since we now have to admit that the true slope might in future prove flatter than in the past by an unknown amount. If we exclude this possibility, and assume as above that the true slope β will remain fixed at 16·3, it would appear from the following idealised examples (in which the dosage ratios and percentage responses have been chosen as arithmetically convenient) that the decision to interpret each assay as a self-contained one entails, if it is to retain the desired degree of accuracy, an average increase in size of up to 50 per cent.—

(i) If the true slope β is known to be 17·03, $P = 0{\cdot}95$ limits of ±10 per cent. will be obtained by using a total of 58 animals, provided that all doses administered lie within the 25 to 75 per cent. response range.

(ii) If we interpret as a self-contained experiment a four-point assay with equal dosage groups in which the dose-ratio is 0·89 : 1 and the proportions responding to the lower and higher doses are respectively one-third and two-thirds, the estimated slope is 17·02; the fiducial limits ($P = 0{\cdot}95$) are ±9·5 per cent. if the test has been carried out with 84 animals, and ±7·0 per cent. with 96 animals.

(iii) Similarly, if the dose-ratio is 1·0 : 1·2 and the proportions responding are one-quarter and three-quarters, the estimated slope is 17·04; the fiducial limits ($P = 0{\cdot}95$) are then ±10·9 per cent. with a total of 80 animals, and ±9·4 per cent. with 96.

(iv) If it is desired to test the linearity of the log dose - probit curves, we can in place of the four-point assays considered in (ii) and (iii) adopt six-point designs with dose-ratios 0·89 : 0·94 : 1 and 1·0 : 1·1 : 1·2 approximately, keeping the total numbers of animals at 96 by using for each preparation dosage-groups of sizes 21, 6, 21 and 20, 8, 20 respectively. If the central groups give 50 per cent. response, as they ideally should, the limits ($P = 0{\cdot}95$) will be ±8·8 per cent. in the former case and ±9·8 per cent. in the latter.

The steepness of the slope in the assay methods under discussion implies that an accurate result can be obtained from a relatively small-scale test; but it has the attendant disadvantage that in order to do so the experimenter must know fairly well, before carrying out the assay, what its result is going to be—otherwise he may easily administer a range of doses that will each produce 0 or 100 per cent. response. One possible method of avoiding this difficulty is to carry out a preliminary pilot assay, using small dosage-groups and covering a wide range of doses, in order to locate roughly, for each preparation, the effective portion of the dosage range. As an alternative, which we hope to discuss more fully on some subsequent occasion, it should be possible to adopt in a comparative assay the sequential procedures developed in recent years for the economical assessment of the sensitivity of explosives.[7,8]

SUMMARY

1. If a solution of *d*-tubocurarine of suitable concentration is administered intravenously to a number of mice a proportion of them temporarily lose the righting reflex.

2. Analysis of a number of experiments indicates that the log dose - probit line obtained by administering intravenous doses at several levels to groups of mice is both steep and straight. This response may be made the basis of an assay method.

3. Analysis of a number of experiments indicates that the log dose - probit line obtained in a similar way with *d*-OO-dimethyltubocurarine in rats is also steep and straight and that this response may also be made the basis of an assay method.

4. The relation of these results to those of previous authors, the convenient form of assay, and the merits of the mouse and the rat for assay of *d*-tubocurarine and its dimethyl ether are discussed.

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Assay of Curare Preparations by the Rabbit Head-drop Method

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A RABBIT head-drop method for standardising curare preparations was first used by Holaday in America about 1940, but so far as we are aware no description of his procedure has yet been published, except a sentence or two in a paper[1] on the use of curare to soften therapeutic convulsions. Modifications of Holaday's test have been briefly employed by Chase, Smith and Bhattacharya[2] and by Everett.[3] We describe below a method of assay based on the same principle, which has been found convenient for checking the potency of *d*-tubocurarine preparations and for assessing the activity of new curarising compounds (*cf.* Barlow and Ing,[4,5] Paton and Zaimis[6]).

PRINCIPLE—A solution of the drug is infused at a steady slow rate into the ear vein of a rabbit until the animal is so weakened that it can no longer hold up its head. The volume of solution which must be injected to produce this end-point is noted. The unknown preparation is compared with the standard in a two-day cross-over test.

APPARATUS—We have used a simple apparatus, made for us by Dr. B. Wheeler Robinson, which enables us to treat four rabbits simultaneously. The barrels of four 5-ml. syringes, lubricated with liquid paraffin and containing the solution to be injected, are clamped in a rack and their plungers are attached to a movable carriage, which is propelled along guide rails by a gramophone motor and reduction gearing. The displacement of the plungers is measured by means of a Veeder counter operated by a cam on the motor spindle. Syringes of nearly the same diameter are chosen, and each of them is calibrated in terms of the counter reading. Repeated calibrations showed that the volume injected could be measured with an error of less than ±0·5 per cent. In most of our tests the infusion rate was 0·4 ± 0·01 ml. per minute, and the volume infused per rabbit 3 to 5 ml. Small variations in the speed of infusion are unimportant so long as the total volume injected can be measured accurately.

The rabbits sit side by side in wooden stalls fitted with wire yokes (Fig. 1). Their heads, which project over the table edge, can move freely up and down, but not far in any other direction. Each rabbit occupies the same stall on the two days of the test and receives the drug from the same syringe. The needle in his ear vein is connected by polythene tubing (2-mm. bore) to the syringe and is kept in place by a bulldog clip fixed just peripheral to the puncture; a strip of adhesive tape binds the plastic tubing to the tip of the ear. The plastic tubing is less distensible than rubber tubing; its capacity is hardly altered when it is coiled or straightened and its transparency makes it possible to ensure that no air bubbles are present. Light-weight buttless needles with a short bevel, such as those of the Solila pattern ($\frac{7}{8}$-inch: Amalgamated Dental Co.), are most satisfactory.

PROCEDURE—The rabbits having sat for 10 minutes or more in their stalls, the apparatus is set running and the needles are successively inserted and bound in place. The operator calls out the moment of insertion and an assistant notes the reading of the counter. The rabbit appears normal until about two-thirds of the head-drop dose has been given. The onset of curarisation is usually heralded by a period of restlessness; the pupils may dilate; and the rabbit's head begins to fall, but can be jerked up again. When the true end-point is reached the neck is fully stretched and toneless, and there is no movement of the head in response to a light touch on the snout. At this moment—it can usually be judged within 10 seconds or so—the needle is pulled out, and the counter reading is noted for a second time. From the difference between the counter readings the volume of solution injected is calculated.

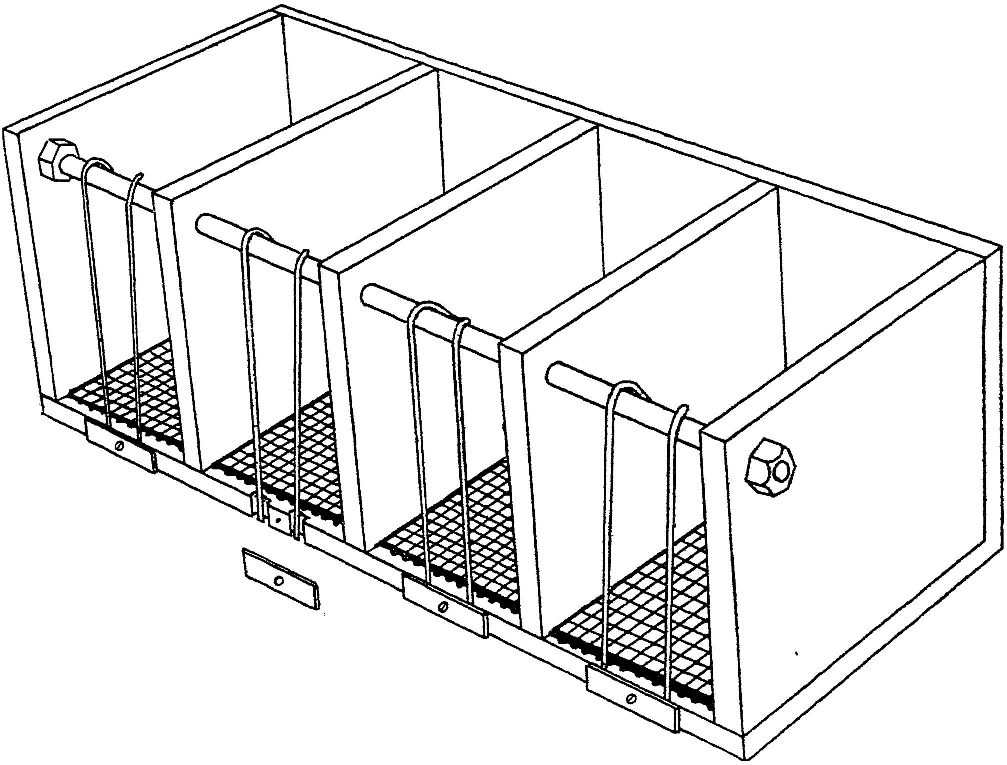

Fig. 1

The rabbits soon recover and seem quite normal 15 minutes later. Occasional animals, especially those unaccustomed to the procedure, suffer severe respiratory embarrassment: they recover quickly if a solution of Prostigmine and atropine (Prostigmine methylsulphate 0·5 mg., atropine sulphate 0·5 mg.) is promptly injected, through the original puncture site, into the vein. It is very important not to excite the rabbits when they are becoming curarised, or after the end-point is reached, because any struggling deepens the curarisation. For this reason docile animals should be selected: we have found both sexes of the Himalayan variety suitable.

On the first day of the test four or more rabbits receive the unknown preparation, and an equal number the standard preparation. The test is completed one to three days later, the groups being crossed over, so that the rabbits that received the unknown on the first day now receive the standard, and *vice versa*.

FACTORS INFLUENCING THE ACCURACY OF THE ASSAY—We have examined the effect of varying the rate of infusion, and the significance of day-to-day and rabbit-to-rabbit variation. In each of two tests on 12 rabbits, three solutions containing 80, 100 and 125 μg. per ml. respectively of *d*-tubocurarine chloride were administered, each rabbit receiving a different solution on each day of the test, according to a replicated Latin square design. The volume of solution injected per minute was kept constant. The variance analysis for one of the tests is shown in Table I. In this test the variance between days was significant, but this was not usual. With rabbits that are accustomed to the procedure there is usually no significant increase or decrease in sensitivity with repetition of the treatment. Untrained rabbits, or those that have not been used for some weeks, are apt to be more resistant to tubocurarine, but a single test increases their sensitivity to a level which is maintained with minor fluctuations through succeeding tests. It is best if all the rabbits in a cross-over assay have been tested within the preceding week; if fresh rabbits have to be used, they should be divided equally between the two groups.

TABLE I

SOURCES OF VARIATION IN THE HEAD-DROP DOSE: ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom, DF	Mean square	F	P
Infusion rate:				
Slope	1	15·0416	—	
Linearity	1	833·6805	3·03	> 0·05
Days	2	1344·5278	4·89	> 0·01 < 0·05
Unconfounded interaction	2	264·4456	—	—
Between rabbits ..	11	2318·6566	8·44	< 0·001
Within rabbits	18	274·8519		
Total	35	34529·2222		

Variance between rabbits is highly significant, whether dosage is recorded on a body-weight basis or directly, and the cross-over design is therefore necessary for maximum efficiency.

The curarising dose is nearly independent of the time taken to administer it unless the time varies widely, as is shown in this test by the insignificance of the variance due to change of infusion rate. The average time required to curarise was here 7 minutes for the strongest solution and 12 minutes for the weakest solution; and within these limits differences in the mean duration of the infusion as between standard and unknown solution can probably be disregarded with safety. With infusion rates much slower or faster than these the amount of tubocurarine required to produce the end-point is somewhat increased (Fig. 2): with a slow injection because an appreciable part of the dose has been inactivated before the injection is completed, and with a fast injection because the drug does not become fully effective immediately on entering the ear vein, so that the curarisation continues to get deeper after the end of the infusion.

CALCULATION OF RESULTS—The dose of each preparation given to each rabbit is recorded in mg. (or ml.). The potency ratio and its limits of error are then conveniently calculated as shown in Table II, where $n/2$ is the number of animals per group, and the standard is administered on the first day to group A and on the second day to group B.

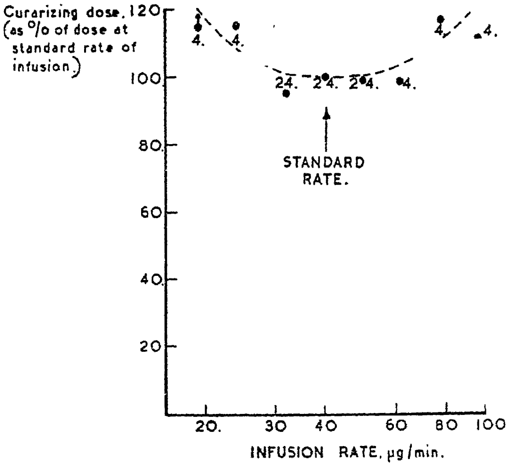

Fig. 2

It will be noted that with this treatment the variance between days does not contribute to the error. It is assumed that the curarising dose is independent of the time taken to administer it and, as has been seen, this assumption is legitimate provided that the mean infusion time is not too different for the two solutions. Where the mean times with standard and unknown differ by more than 20 per cent. it is wise to repeat the test.

EXAMPLE—Table III gives the data obtained in a typical cross-over test, in which two preparations of *d*-tubocurarine chloride were compared. Each preparation was administered in the form of a 0·01 per cent. solution in 0·9 per cent. sodium chloride solution and the infusion rate was 0·4 ml. per minute.

TABLE II

	Rabbit No.	Log doses		Differences
		Day 1	Day 2	$(X_s - X_u = x)$
Group A ..	1	$X_{s(1)}$	$X_{u(1)}$	x_1
	2	$X_{s(2)}$	$X_{u(2)}$	x_2
	3	$X_{s(3)}$	$X_{u(3)}$	x_3
	⋮	⋮	⋮	⋮
	⋮	⋮	⋮	⋮
		(Mean for Group A) ..	..	$\bar{x}_A$
Group B ..	⋮	⋮	⋮	⋮
	⋮	⋮	⋮	⋮
	$n-2$	$X_{u(n-2)}$	$X_{s(n-2)}$	x_{n-2}
	$n-1$	$X_{u(n-1)}$	$X_{s(n-1)}$	x_{n-1}
	n	$X_{u(n)}$	$X_{s(n)}$	x_n
		(Mean for Group B) ..	..	$\bar{x}_B$

$$M = \text{log potency ratio, unknown : standard} = \tfrac{1}{2}(\bar{x}_A + \bar{x}_B)$$

$$VM = \text{variance of } M = \frac{S(x - \bar{x}_A)^2 + S(x - \bar{x}_B)^2}{n(n-2)} = s_M{}^2$$

Limits of error of M are $M \pm ts_M$ with t for $(n - 2)$ degrees of freedom.

TABLE III

	Rabbit No.	Weight kg.	Curarising dose (μg.)			
			Standard		Unknown	
Group A	1	2·0	287	on Day 1	276	on Day 2
	2	1·8	285		303	
	3	2·0	286		314	
	4	1·7	341		378	
Group B	5	1·7	294	on Day 2	312	on Day 1
	6	1·8	311		320	
	7	1·9	391		315	
	8	1·8	443		401	

These results, when treated by the method already described (Table II), give the following results—

Potency (unknown : standard) ..	1·001
Limits of error (P = 0·05)	0·917 and 1·093

ACCURACY OF THE ASSAY—From the data of the Latin square tests it was calculated that if 8 rabbits were used in a cross-over assay the limits of error (P = 0·95) of the potency ratio, determined by the formula given above, could be expected to lie within ±10 per cent. This expectation has been verified in 10 out of 13 routine assays of this sort; in the other 3 the fiducial limits were slightly wider. In 10 consecutive assays of known solutions of *d*-tubocurarine chloride the largest error in estimating the mean potency ratio was 8 per cent.

CHOICE OF METHODS FOR THE ASSAY OF CURARE PREPARATIONS—We think that this depends, to some extent, on the sample to be examined. If the material purports to be, for instance, pure *d*-tubocurarine chloride, and has to be compared with an authentic specimen of the salt in order to check its freedom from impurities of higher or lower activity, then it probably matters little which method is chosen, so long as it is convenient and gives reproducible results. If the material to be tested is an impure preparation that is supposed to owe its activity to a particular alkaloid, there may be some advantage in the use of an *in vivo* rather than an *in vitro* method of comparison. It is possible that surface-active material or other impurities might affect the rate of curarisation when the drug is applied directly to the muscle, whilst having less effect when the drug has to be transported to the muscles by the blood; though we have no evidence that this possibility is realised in practice. In any event there is something to be said for having the whole animal under observation and not merely a piece of muscle, particularly where one is testing a preparation intended for clinical use. It must be remembered, however, that the impurities may also be toxic: neither death, head-drop nor immobilisation is a specific test for curarising activity. Within these limitations the rabbit head-drop procedure has the advantages of being simple to carry out and of producing data from which the precision of the result can be calculated.

In comparisons of the kind referred to above it is presumed that the activity being measured is due to a single active principle. When new curarising compounds are examined an attempt is made to state their potency in terms of a reference substance. Here it is certainly too early to name any single test as pre-eminently reliable for predicting potency in clinical application. Recent work[4,5,6] has emphasised the remarkable variability in the relative activity of curarising compounds, depending on the species and the muscle used for the comparison. Until the significance of these discrepancies is better understood it seems wisest to recommend that whatever screening test be used the most active compounds at least of any series should be tested on more than one muscle or more than one species.

SUMMARY—A procedure is described for the assay of curarising drugs by means of the head-drop reaction in the rabbit. The error of a cross-over test on eight animals is usually less than ±10 per cent. (limits for $P = 0{\cdot}95$).

We are indebted to Dr. C. W. Emmens for statistical advice, and to Mr. C. Pergande for technical assistance. Permission to administer curarising drugs to unanaesthetised animals was granted by the Home Office.

One of us (N. K. D.) is grateful to Sir Charles Harington, F.R.S. for permission to work at the National Institute for Medical Research, and to the Government of India for a grant.

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Use of a Rotating Drum in Assessing the Activities of Paralysant, Convulsant and Anaesthetic Drugs

BY H. O. J. COLLIER, R. A. HALL AND E. C. FIELLER

(*Read at a meeting of the Biological Methods Group, May 26th,* 1949)

THE assay of curarising activity by visual observation of the righting reflex, described in a preceding communication,[1] although rapid, simple and in our opinion reliable, suffers from the defect that it depends upon a subjective judgment. We have therefore devised a rotating drum apparatus in order to render objective the decision whether or not a certain degree of paralysis has been effected in a mouse.

The objects of the present paper are—

(1) To compare tests of curarising activity carried out with the rotating drum with those based on visual observation of the righting reflex; and

(2) To describe the use of the drum in estimating the activities of various compounds, namely *d*-tubocurarine, decamethonium iodide and its relatives, "myanesin," thiopentone and leptazol.

APPARATUS AND METHODS

The apparatus, which is illustrated in Fig. 1, consists essentially of a 6-inch drum, such as is commonly used for smoked paper recording, arranged so that it can be rotated at a definite speed on a horizontal spindle. The surface of the drum is fitted with wire-mesh to enable mice to grip and run upon it. A smooth wall is fitted on each side of the drum to prevent mice crawling over the edge. A tray of sawdust is placed below the drum in order to catch mice that fall off. Dimensions and specifications of these fittings are given below the figure.

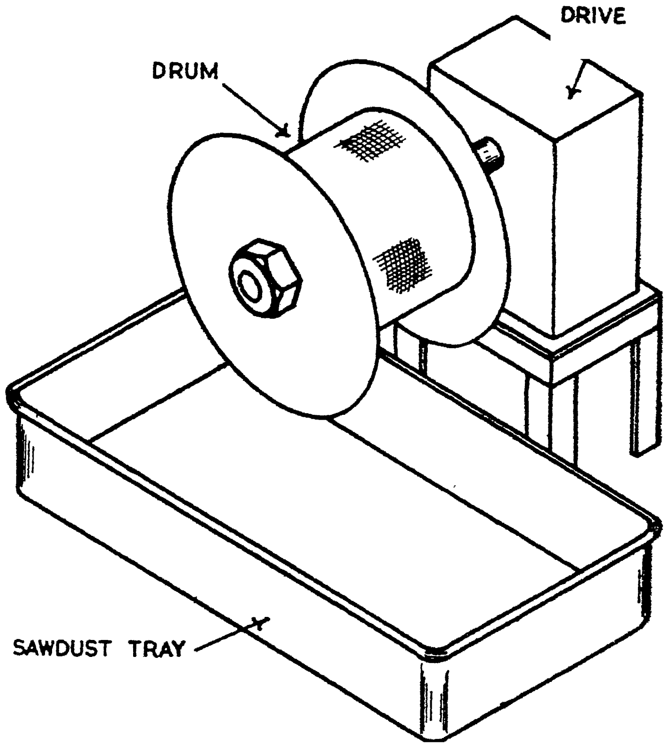

Fig. 1. The cylindrical drum surface, $6\frac{1}{8}$ in. wide and $6\frac{1}{4}$ in. in diameter, is covered with wire gauze of 6 mesh, 21 S.W.G. (0·032 in.) wire. The end walls of the drum are of sheet metal 10 in. in diameter, projecting $1\frac{7}{8}$ in. beyond the wire gauze surface all round, as smooth flanges. The central axis of the drum is mounted $10\frac{1}{4}$ in. above the floor of the sawdust tray. The tray is 20 in. long, $10\frac{1}{2}$ in. wide and 3 in.

In order to find the optimal speed of rotation the drum was connected to a motor by two pulley systems through a reducing gear box. Investigation of the behaviour of normal mice on the drum, rotating at speeds ranging from 2 to 10 r.p.m., showed that a speed of 2·1 r.p.m. (corresponding to a surface speed of 19 mm. per second) was satisfactory for our purposes. At this speed many mice will continue to run round the drum for half an hour or more.

In order to observe the behaviour of saline-treated mice upon the drum, 100 animals were injected intravenously in turn with 10 ml. of normal saline per kg. of body weight. Each was placed on the drum immediately after injection and allowed to remain on it for 3 minutes. Of these 100 animals, 92 ran against the direction of rotation of the drum and retained their hold for the full period; 8, on the other hand, allowed themselves to be carried round on the moving surface and leaped from the drum into the sawdust tray below. It is therefore necessary to take precautions, which will be described below, against counting such mice as positive reactors in a test.

The routine of performing a test on the rotating drum was in most respects similar to that described previously.[1] After intravenous injection of the drug the mouse was immediately placed on the upper surface of the rotating drum, facing against its direction of movement. Those mice that were unable, through the action of the drug, to retain their hold on the moving surface, slid off and fell into the tray below. These were counted as positive reactors. Those that, in spite of the administration of the drug, remained on the drum for longer than the time taken for the action of the drug to pass its peak (up to 3 minutes), were counted as negatives. The occasional mouse that 'stayed put' on the surface, rotated with the drum and leaped into the tray could quite readily be distinguished from the affected mouse that slid off from the upper part of the surface. Any doubts on this point were settled by putting the mouse in question once more on to the drum.

RESULTS

Responses to curarising drugs—As might be expected, administration of adequate doses of the drugs listed in Table I, which have a curarising action, prevented a mouse from retaining its hold on the drum surface. Intravenous administration of a drug at various dosage levels to groups of mice, followed by observation of the ability of each animal to retain its hold on the drum, provided data which could be submitted to the customary routine of plotting the dosage - response curve, testing its linearity and estimating its slope, the ED 50 and its fiducial limits. Tests of linearity showed the log dose - response lines obtained with each compound in Table I to be satisfactory.

TABLE I

MEAN SLOPES OF LOG DOSE - PROBIT RESPONSE LINES OBTAINED WITH THE ROTATING DRUM AND BY OBSERVATION OF THE RIGHTING REFLEX IN MICE, USING FOUR SUBSTANCES HAVING CURARISING ACTIVITY

	Rotating drum method			Righting reflex method		
Drug	Total No. of mice	Mean slope	Standard error	Total No. of mice	Mean slope	Standard error
d-Tubocurarine chloride ..	110	17·6	± 3·7	662	16·3	± 2·0
Decamethonium iodide	260	13·7	± 1·8	235	10·8	± 1·6
Hexamethonium iodide	40	15·2	± 6·0	40	17·4	± 7·8
Pentamethonium iodide	220	16·3	± 2·4	20	33·9	± 15·0

The question whether the rotating drum method provides a measure of activity more accurate or less accurate than observation of the righting reflex can be answered by comparison of the slopes obtained with these two methods of estimating the degree of paralysis. It will be seen that, for any one compound, there is no significant difference in the slopes obtained by the two methods. For practical purposes, however, the rotating drum is to be preferred, since it does not require trained judgment.

The relative sensitiveness of our two methods of estimating paralysis provides a second point of interest. With all curarising compounds for which this point has so far been investigated it has been found that a positive reaction on the rotating drum may be obtained at a lower dosage level than is required to abolish the righting reflex. This point is illustrated in Fig. 2, in which the ED 50 for decamethonium iodide obtained with the rotating drum was in one experiment 83, and in another 91 per cent. of the ED 50 required to abolish the righting reflex.

Fig. 2 also illustrates the point mentioned in our previous paper (p. 583), that the position of the ED 50 may vary from experiment to experiment, although the slope of the curve does not normally do so significantly.

If fiducial limits are calculated directly from the data of these assays, chance variations in the observed value of the slope will of course have a considerable effect on the values obtained, although the true slope is constant. The example illustrated in Table II, which is based on the assumptions that the value found for the slope will be 15 and that the percentages of animals responding in the different dosage groups will approximate to 20 and 80, may serve as a guide to the accuracy to be expected.

Responses to other drugs—The question arises how far the rotating drum method may be used for the estimation of substances other than those with curarising activity. One limitation is obviously imposed by the present form of the apparatus; the compound must be one to which the animal can respond fairly rapidly. For this reason, and for another stated in the previous paper, we believe that the intravenous route of administration of a drug should be used.

Provided the substance can be administered intravenously we might expect that drugs impairing nervous co-ordination in various ways would give a positive response in mice on the rotating drum. Accordingly we obtained trial dose-response curves with intravenous "myanesin," thiopentone and leptazol. Each of these curves, which are illustrated in Fig. 3, are steep and rectilinear.

TABLE II

EXAMPLE OF THE RELATION OF FIDUCIAL LIMITS TO NUMBERS OF MICE EMPLOYED

Fiducial limits (P = 0·95)	No. of animals dosed with standard preparation	No. of animals dosed with test preparation
± 10%	40	40
± 7·5%	70	70
± 5%	150	150

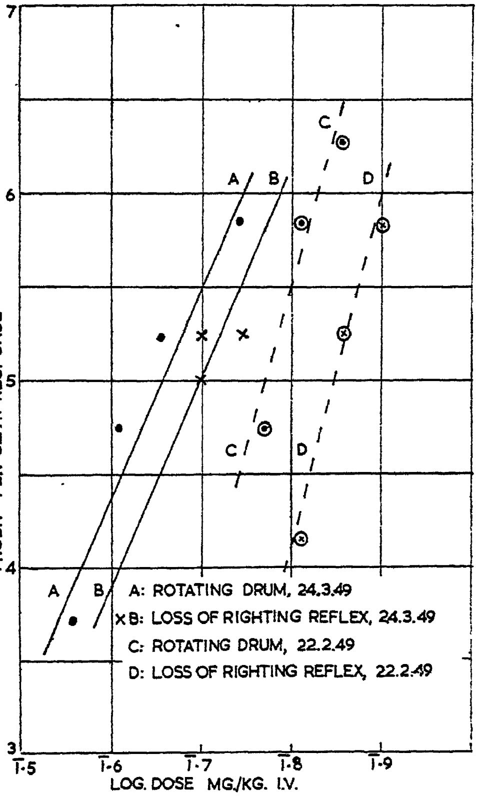

Fig. 2. Log dose - probit response lines obtained with a sample of decamethonium iodide on two different days. On each occasion the loss of righting reflex and the ability to hold on to the rotating drum were noted

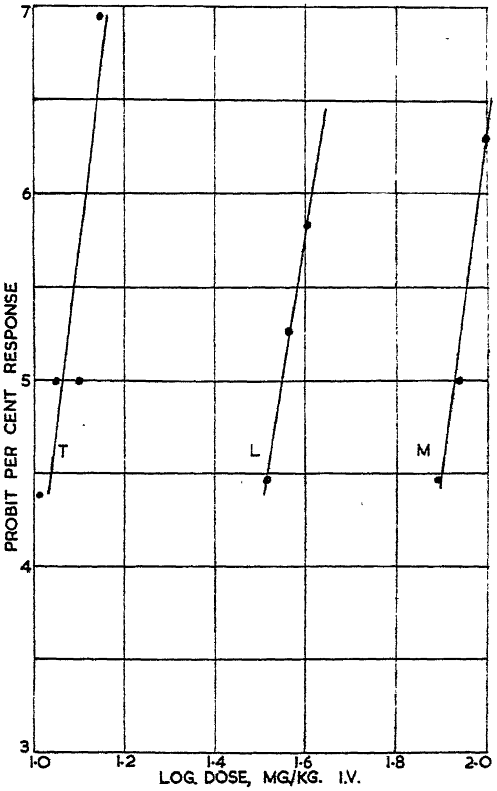

Fig. 3. Log dose - probit response lines obtained with Thiopentone (T), Leptazol (L) and "Myanesin" (M) by means of the rotating drum. Ten mice were used at each dosage level

Although we have not performed a sufficient number of experiments to draw a final conclusion, our results suggest that the type of drum described may be used for estimating convulsant and anaesthetic, as well as paralysant substances.

SUMMARY

1. A rotating drum apparatus, which may be used for estimating the degree of paralysis of a mouse after intravenous administration of a curarising drug, is described.

2. The results obtained with some substances having curarising activity, namely deca-, hexa- and penta-methonium iodides and *d*-tubocurarine chloride, are reported.

3. Comparison of the dose - response curves obtained with the drum and by observation of the righting reflex show that there is little difference in accuracy between the two methods of measuring degree of paralysis.

4. Mice on the rotating drum respond to a somewhat slighter degree of paralysis than is required to abolish the righting reflex. The drum method is therefore more sensitive.

5. Satisfactory dose - response curves have also been obtained with the drum after intravenous administration of other drugs impairing nervous co-ordination, namely "myanesin," thiopentone and leptazol.

We should like to thank the Directors of Messrs. Allen and Hanburys, Ltd., for permission to publish this work, Miss J. E. Pryke for assistance in biological experiments and Mr. A. Marshall for drawing Fig. 1.

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DISCUSSION ON THIS PAPER

Dr. M. R. A. **CHANCE** asked whether the exceptional behaviour of 8 per cent. of the mice could arise from the fact that many rodents in captivity do not obtain sufficient exercise in scratching to keep their claws to the normal length, and Mr. A. L. **BACHARACH** enquired whether it was always the same individual mice that behaved differently. Dr. **COLLIER** replied that the anomalous mice were characterised by unwillingness to run on the drum rather than by inability to hold on to the surface. Mr. **HALL** pointed out that the wire of the mesh was too thick in gauge to permit the mice to enclose it completely in their claws. He added that normal mice that had fallen off the drum would remain on when replaced.

Dr. **CHANCE** also asked whether the effect of leptazol, which caused the mice to fall off the drum, appeared at dose levels much below that necessary to produce obvious signs of central nervous stimulation, such as convulsions. Mr. **HALL** said that this was so.

Finally, Dr. **CHANCE** asked whether the relative activities of a substance tested by the rotating drum and righting reflex methods would give information on the different dose required to stimulate different parts of the central nervous system. Dr. **COLLIER** replied that as the different peripheral actions of curare on the neuro-muscular junctions of the digits and of the limbs might be studied by the method suggested, he thought the same might apply to drugs that had an action on different parts of the central nervous system at different dose levels.

Mr. R. F. **SELLAR** suggested that some of the "unco-operative" 8 per cent. of mice might be deterred from jumping voluntarily from the revolving drum by raising the latter and Dr. G. A. **MOGEY** pointed out that he had not found this manoeuvre to be very successful. He had been using a horizontal wire grid on which the mice were suspended upside down, and had raised it to a height of 4 feet above the sawdust pit, but some mice still dropped, although the drop did not appear to have been occasioned by the tubocurarine. He described how mice tended desperately to cling on to the grid with their fore-paws after they had relaxed the grip of the hind paws as the action of the paralysing agent asserted itself—whereas when about to jump off deliberately they let go first with their fore-paws and groped about in the air with them as if searching for a suitable landing place. In conclusion, Dr. **MOGEY** reminded the meeting of the necessity of paying very strict attention to the point that curarised animals must not be hurt.

Dr. G. E. **FOSTER** asked whether Dr. Collier had experienced any difficulty in carrying out tests on *d*-tubocurarine preparations so that the results would conform with the limits required by the Therapeutic Substances Act. Dr. **COLLIER** replied that the precision attained depended on the number of animals used: a large number was required to conform with the T.S.A. limits.

The Extraction of Growth Factors from Natural Products prior to Microbiological Assay

BY J. S. HARRISON

(Read at the Annual General Meeting of the Biological Methods Group, December 17th, 1948)

THE methods used for the extraction of growth factors of the vitamin B complex from their bound forms in natural products are essentially empirical. Treatments with acid, alkali or enzyme preparations are usually employed and the highest valid result from a particular material is taken as the best available value. The extraction of pantothenic acid and the vitamin B_6 complex are discussed below.

PANTOTHENIC ACID

The accepted method of extracting pantothenic acid from natural products is a combination of heat and enzyme treatments.[1,2] Until recently the following technique was used in this laboratory for dried yeast and other products prior to assaying with *Lactobacillus arabinosus*—

> Steam 1 g. of material in 25 ml. of 0·1 *M* sodium acetate buffer solution for 30 minutes at pH 6·8, cool, and incubate at pH 4·5 for two days at 37° C. with 0·5 g. each of papain and takadiastase of known pantothenic acid content.

Recently we found that in certain cases, notably with malt extract, a simple treatment with 1·67 *N* sodium hydroxide for 5 minutes at room temperature would release two and a half times as much pantothenic acid activity as the orthodox enzyme treatment. This high value was not increased by further enzymic treatment. Malt, on the other hand, gave a value of 5 µg./g. by enzyme treatment, which was increased to about 10 µg./g. by sodium hydroxide and further to 15 µg./g. by a combination of alkali and enzyme treatments. Malt wort and various other cereal products showed similar increases. Optimum conditions for extraction were found by experiment and the standard treatment finally adopted is given below. Control experiments with calcium D-pantothenate showed that under these conditions less than 5 per cent. of the activity was destroyed by the alkali.

> Dissolve or suspend 1 to 5 g. of material, according to its expected pantothenic acid content, in 25 ml. of 0·1 *M* sodium acetate at pH 6·8, and steam for 30 minutes. Cool, add 5 ml. of 10 *N* sodium hydroxide and allow to stand for exactly 5 minutes, then add 5·1 ml. of 10 *N* sulphuric acid or its equivalent and adjust to pH 4·5 by addition of sodium hydroxide. Add exactly 0·5 g. of papain and 0·5 g. of takadiastase, both of known pantothenic acid content under the conditions of the test, cover with a layer of toluene, incubate at 37° C. for 3 days, filter (or preferably centrifuge if filtration is difficult), and make up to a suitable known volume from which the assay dilutions can be prepared. Extract a portion of the dilution twice with light petroleum before testing, unless this treatment has been proved to be unnecessary, as the effect of certain fats on the growth of *L. arabinosus* is considerable.

In assays for which less than the complete treatment was necessary, the appropriate stage in this schedule was omitted. An interesting point which may have some connection with the effect produced by the alkali was that, almost invariably, the soda plus enzyme treatment gave a perfectly clear solution, whilst the enzymes alone and soda alone gave solutions that were difficult to filter and remained cloudy.

Table I gives the results obtained with various materials. Heart muscle is included on account of its high content of co-enzyme A. The greatest increases in apparent pantothenic acid content were obtained with malt products. With malt itself the maximum liberation was obtained with soda plus enzymes, whilst for malt extract it was sufficient to use soda only. This can probably be explained by prior action of the natural enzymes on the raw materials, and it is not surprising that additional incubation with further enzymes does not liberate any more of the growth factor. As the apparent pantothenic acid content of heart muscle was not significantly increased, it was concluded that co-enzyme A is not affected by cold alkali treatment.

All the values so far quoted were obtained with *L. arabinosus* as the test organism. In a smaller number of tests the same effects were observed in using the *Saccharomyces carlsbergensis* assay. As examples of the similarity of the results, a particular sample of malt extract gave, untreated and soda-treated respectively, 6·5 μg./g. and 19·2 μg./g. by the lactic assay and 5·0 μg./g. and 20·4 μg./g. by the yeast method. The large increase which has been obtained over the previously accepted values makes previous figures for pantothenic acid balances in malt wort fermentations meaningless.*

VITAMIN B$_6$

The microbiological assay of the vitamin B$_6$ group has received considerable attention recently, and there have been interesting developments in respect of the extraction and the different behaviour of the various members of the complex.

TABLE I

PANTOTHENIC ACID ACTIVITY (μg./g.) OF SOME NATURAL PRODUCTS

	Method of extraction			
Sample	Water	Enzymes	Soda	Soda + Enzyme
Malt	3·0	5·4	10·3	14·6
Malt extract	7·8	9·0	25·2	25·1
Grain wort	0·4	0·9	—	1·2
Wheat germ	—	19·4	19·9	19·0
Cane molasses	8·0	8·4	9·0	9·4
Dried bakers' yeast	66	111	65	116
Sheep's heart	—	27·7	—	32·9
Mutton	—	14·1	—	13·1

Acid extraction—In the early published work on this subject alkaline hydrolysis was rejected presumably because of the known destruction of vitamin B$_6$ by alkali, and heating with mineral acid became the standard treatment. In the early acid extractions, when *Neurospora sitophila* mutant 299 was used as the test organism, *N* hydrochloric acid was used,[3] 1 to 5 g. of the sample being heated with 40 ml. of *N* hydrochloric acid. In 1947, Rubin, Scheiner and Hirschberg,[4] using sulphuric acid in the extraction of dried brewer's yeast, demonstrated that the efficiency of extraction of vitamin B$_6$ activity for *S. carlsbergensis* during an extraction time of 1 hour at 20 lb. pressure, was greatest in the relatively narrow range of pH 1·5 to 2·0, and diminished progressively on either side of these limits. The lower response of the test organism to extracts prepared at other pH levels could be raised to maximal or near-maximal by further hydrolysis in 0·055 *N* sulphuric acid for 8 hours, or by enzymic treatment. Similar release of activity was shown for vitamin B$_6$ in liver. Since the publication of Rubin, Scheiner and Hirschberg, other papers have been presented to explain the chemical mechanism involved in this phenomenon. It is related to the presence of pyridoxamine phosphate in natural products such as dried yeast, as demonstrated by Rabinowitz and Snell.[5] The same workers showed that the acid extraction could be improved considerably by extending the 1-hour hydrolysis to 5 hours.

It was shown by Atkin, Schultz, Williams and Frey[6] that the optimum pH for liberating vitamin B$_6$ from wheat or wheat products was lower than for yeast and many other materials. We have confirmed this, and in experiments in our laboratories with dried baker's yeast and two different types of molasses, in which 100 mg. of the sample were autoclaved for 2 hours at 20 lb. pressure with 25 ml. of sulphuric acid of various strengths, the optimum normality for yeast was shown to be 0·055 (pH 1·7), whereas that for molasses was 0·5 (pH 0·7).

Extraction by enzymes—The efficiency of a method of extraction can be assessed by comparing the results with those obtained by a second method. Rabinowitz and Snell[7] used an enzyme preparation, clarase, for the extraction of vitamin B$_6$. Using this method we first worked out the best conditions for extraction with dried baker's yeast as the test substance. Maximum extraction was obtained in 3 days when 100 mg. of dried yeast,

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* Since this paper was read a communication by Neilands and Strong (*Arch. Biochem.*, 1948, **19**, 287) has appeared that describes an animal enzyme technique by which the apparent pantothenic acid content of various natural products can be considerably increased. Among the substances tested was dried yeast, the pantothenic acid content of which was increased fourfold by the new treatment, whereas alkali treatment has proved to be without appreciable effect.

100 mg. of clarase of known B_6 content, and 10 ml. of *M* sodium acetate at pH 5, covered by 2 ml. of sulphur-free toluene, were incubated at 37° C. With this technique good agreement was obtained between the enzyme and acid extractions. Actual values are given in Table II.

TABLE II

COMPARISON OF ACID AND ENZYME EXTRACTION OF VITAMIN B_6 ACTIVITY FROM YEAST AND MOLASSES

Sample	Acid extraction 5 hr. at optimum pH	Clarase extraction 3 days at 37° C.
Dried bakers' yeast	[0·055 *N*] 20·3 µg./g.	20·7 µg./g.
Beet molasses	[0·5 *N*] 5·57 „	5·25 „
Cane molasses	[0·5 *N*] 11·6 „	11·4 „

The foregoing results were obtained with the Carlsberg yeast assay. Substantially similar results were obtained in a shorter series of experiments with *N. sitophila* mutant 299.

Available vitamin B_6—The ideal method of determining the amount of available growth factor in a natural product is to carry out assays under the precise conditions to be studied. This is often impossible where large-scale processes are involved, but the assay method should be designed to approach as nearly as possible to the practical conditions. In a study on these lines of the availability to yeasts of the vitamin B_6 complex in molasses, it was found that the amount of available growth factor measured by microbiological assays with two different yeasts gave different values. This is understandable because different organisms are known to vary in their capabilities of utilising bound or modified forms of growth factors. It followed that more of a factor might become available to the same organism with increased time of growth, if cell enzymes took an appreciable time to attack the bound factor, or if the growth had to reach a certain value before a sufficient concentration of enzyme was reached. Hence the apparent B_6 content of molasses might increase with longer incubation time of the assay. Two strains of *S. cerevisiae* and one of *S. carlsbergensis* were tested, with the surprising results that the values obtained with *S. carlsbergensis* decreased with time, whereas the *S. cerevisiae* values increased, but to a figure much higher than the maximum obtained by acid hydrolysis. No similar variation in results with time of incubation was found when acid-hydrolysed samples were used. It would appear that values higher than the "complete" figure must be invalid as a measure of the true B_6 complex, although no great "drift" was observed at various levels. No satisfactory explanation of this phenomenon is yet forthcoming.

TABLE III

EXTRACTION OF GROWTH FACTORS FROM NATURAL PRODUCTS

	Method of extraction		
Growth factor	Enzymes	Acid	Alkali
Vitamin B_6	Maximum with clarase	Maximum at correct pH	Sub-maximum
Pantothenic acid ..	Maximum with animal enzyme*	Destroyed hot	Destroyed hot, maximum cold, in certain cases with enzymes
Biotin	Sub-maximum	Maximum 126° C. 3–6 *N* H_2SO_4, 1 hr.	—
Inositol	—	Maximum 100° C. 6 *N* HCl, 20 hr.	—
Aneurine	Maximum with prior acid treatment	Maximum 100° C. when followed by enzymes	Destroyed
Nicotinic acid	Mostly sub-maximum	Maximum 120° C. *N* HCl, 20 min.	Maximum for cereals
Riboflavine	Useful for tissues	Maximum 120° C. 0·25 *N* HCl, 15 min.	Destroyed

* See Neilands, J. B., and Strong, F. M., *Arch. Biochem.*, 1948, **19**, 287.

CONCLUSION

In Table III a summary is given of the methods of extraction that have been found to be most satisfactory for the liberation of members of the B complex of growth factors from natural materials. The table only represents general findings and may need considerable modification as more experimental results become available. In the case of pantothenic acid, two improved methods are immediately introduced. It is, in any case, quite certain that any new test substance must be examined under various conditions to find the most suitable extraction technique. At present no general method is satisfactory for all materials because the forms of the chemical combinations within the living cell are not understood. Until these problems are solved empirical procedures must, of necessity, be used for the extraction of growth factors from natural products.

SUMMARY

A method is described for the extraction of pantothenic acid from natural materials by treatment with cold alkali which gives considerably higher values for the pantothenic acid content of malt products and other substances than the orthodox enzyme treatment. Methods of estimating vitamin B_6 in both free and bound forms are discussed.

The author thanks the Directors of the Distillers Company Limited for permission to publish this paper.

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DISCUSSION

Dr. A. J. AMOS congratulated the author on his interesting paper. The method of extraction of test materials, he said, was an aspect of microbiological assay that did not always receive sufficient attention.

Dr. MORRIS stated that in his experience the use of *N* NaOH for the extraction of pyridoxine caused no loss of the vitamin at all and the method could be used for assay purposes. Subsequent treatment with sulphuric acid gave no increased response.

Mr. S. A. PRICE said that in his experience a frequent result of unsuitable extraction procedures for pantothenic acid was non-valid assays in which the liberation of the vitamin could not be evaluated precisely. Did Mr. Harrison not have this difficulty, or was he using a better medium less prone to give such troubles?

Mr. HARRISON replied that he used one of the published media for pantothenic acid assay, and the validity of the assays was usually satisfactory.

Notes

AN AUTOMATIC BURETTE—VACUUM OPERATED

AUTOMATIC burettes in which a burette is attached to a reservoir and can be filled from it when necessary, are being increasingly used in chemical and other laboratories. Generally they can be divided into two classes. In those intended for use with solutions relatively stable in presence of air the burette is fixed above the reservoir and the solution is pumped into the burette by air pressure, from a blow-ball or rubber bellows. In the other type, which is more often used for solutions liable to alteration of strength by access of air (*e.g.*, solutions of alkali hydroxides, ferrous ammonium sulphate, titanous chloride or sulphate, etc.), the reservoir is kept at a higher level and the solution is fed into the burette near the bottom by means of gravity; and the top of the burette and the reservoir are connected to a source of inert gas. Zintl and Reinacker[1] (1926) designed a special automatic burette for use with solutions unstable in contact with air, and this was further modified by Smith (1935) but it is rather complicated.

In our laboratory we had an apparatus intended for use with a blow-ball, but we wanted our standard solution not to come in contact with air. So we modified it and found that the new apparatus worked more smoothly than the original and had the additional advantage that it could be directly connected with a source of inert gas and used for solutions unstable in presence of air. A diagram of the modified apparatus* is given in Fig. 1.

The apparatus shown, except the connections between the tubes A and C, is one of the several types available on the market. It has a reservoir, R, burette, B, side tubes, A and D, and a tube, S, which narrows down at the extremity N, and acts as a siphon for adjusting the solution in the burette to zero point automatically. Normally a rubber bellows is attached to the point A and a rubber tube with a pinch cock to the tube D. With the help of the rubber bellows, air is blown into the reservoir, R, which forces the liquid into the burette, B; when the pressure in the reservoir is released by opening the pinch cock at D, the liquid near the top of the burette is sucked back into the reservoir till the level is at zero.

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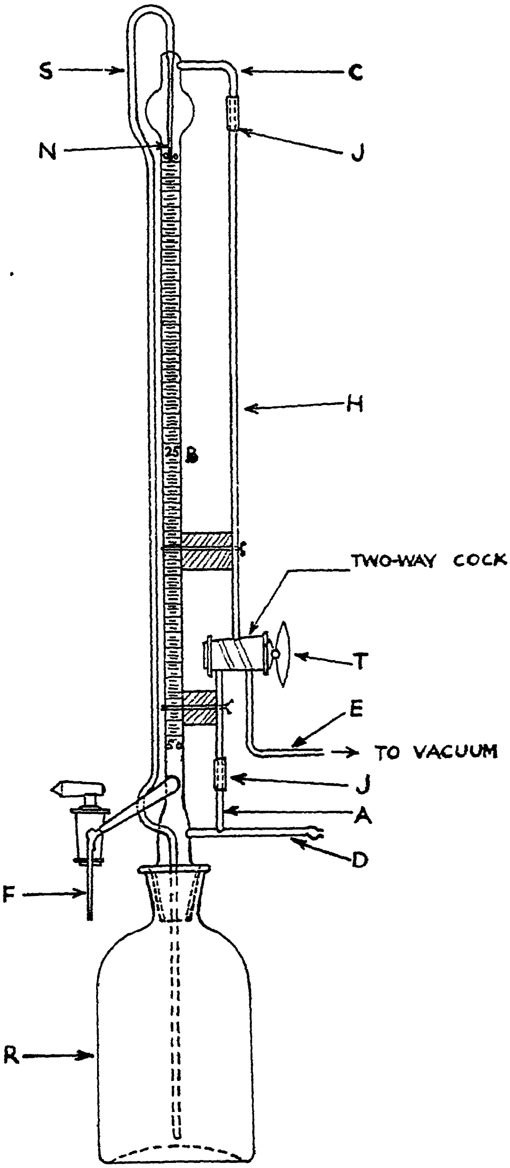
* The design is the subject of a patent application.

For working the apparatus with a vacuum we have connected A to C through a two-way stop-cock T, as shown in the drawing. The joints J and J may be two glass tubes joined by rubber tubing or one continuous glass tube. For use, E is connected to the vacuum. This reduces the pressure in the burette and the solution is sucked up from the reservoir, R, through the tube S. After the burette is filled to above the zero mark, the two-way stop-cock T is turned round to make connection between A and C. Thus both the reservoir and the burette are connected with the atmosphere and the siphon tube S adjusts the level of the solution in the burette to zero. The burette is now ready for use and the solution can be taken from F without any difficulty, as the top of the burette is connected to the amosphere through C, H, A, and D.



For obtaining the reduced pressure, E can be connected to a vacuum line, or a vacuum pump, or air can be merely sucked out by the mouth. The burette can be connected either to the vacuum or to the atmosphere by simply turning the stop-cock T.

For working under an inert atmosphere, D can be connected directly to a small Kipp's apparatus or to a gas cylinder. It is not necessary to have a stop-cock between the Kipp's apparatus and tube D, as the pressure adjusts itself automatically.

For using the apparatus for standard alkali solution, D is connected to a gas washing tower containing a concentrated solution of sodium hydroxide; thus the air entering the reservoir or the burette is always free from carbon dioxide, and there will be no danger of the standard alkali solution being affected.

REFERENCE

1. Vogel, A. I., *Textbook of Quantitative Inorganic Analysis*, 1939.

DEPARTMENT OF CHEMICAL TECHNOLOGY
UNIVERSITY OF BOMBAY

N. B. BHUNVARA
M. L. KHORANA
March, 1949

NOTES ON THE DETERMINATION OF COPPER IN FOODS BY HIGH'S METHOD

THE method described by High[1] for the determination of copper in foods has been found very convenient and reliable. The following comments may be of service to other users of the method.

1. High ashes the sample in a silica basin. We have found that with certain materials, particularly when much phosphate is present, the silica is attacked and retains some of the copper. Experiments in which known amounts of copper were added to a mixture of 50 parts of sucrose and 1 part of potassium dihydrogen phosphate gave from 70 to 92 per cent. recovery of the added copper. A porcelain basin, however, is quite satisfactory as long as the internal glazing is undamaged, provided the temperature of ashing is not allowed to rise above 600° C. Ashing should be continued until the ash is nearly white, but traces of carbon can be filtered off after extraction of the ash without effect on the result.

2. High adds 0·5 g. of his solid reagent (a 1 : 25 mixture of sodium diethyldithiocarbamate and sodium chloride) to an unstated volume of the solution of the ash of the sample. If the total available volume of solution is only 50 ml. (as is necessary when the total copper in the ash is small) the solid reagent may be added to 20 ml. or less, to permit duplication, and we find that 0·25 g. of solid reagent is then amply sufficient for the development of maximum coloration. It is now our practice to take 20 ml. of the solution on all occasions, and economy of reagent is thereby effected.

3. Sodium diethyldithiocarbamate appears to decompose with time even in the solid state, and this is particularly true when ground finely with sodium chloride (perhaps because of the greatly increased surface area). We have found it advisable to purchase small quantities of the reagent frequently, and to keep the mixture with sodium chloride not longer than six weeks. The solutions to which the solid mixture is added must be watched for the slightest sign of turbidity as distinct from coloration; if turbidity is noticed it is almost certainly due to decomposition of the solid reagent. It is advisable to put up a "blank" on the reagents with each set of determinations, and if a previously plotted standard curve is to be used, at least one point on it should be freshly checked. Using the Spekker absorptiometer with Ilford 601 (Violet) filters, and setting the drum reading to 1·0 against the "blank" *without* solid reagent, the reading of the "blank" *with* solid reagent should be not less than 0·98. A solution containing 5 μg. of copper per ml. gives a drum reading of about 0·4.

REFERENCE

1. High, John H., *Analyst*, 1947, **72**, 60.

VIROL LTD.
HANGER LANE, W.5

ERIC C. WOOD
Miss E. M. AULT
March, 1949

LABORATORY INVESTIGATION OF LEAD POISONING IN CATTLE

THE following is a report on the analytical results obtained from the examination of viscera and visceral contents removed from cattle suspected of having been poisoned by ingestion of lead. Reports received showed that the chief offending objects to which cattle gained access were abandoned tins of paint left in fields; pipes that had been re-jointed with red lead were another source of danger. Old military sites re-opened for cattle grazing were also a potential danger unless all metallic refuse had been carefully removed.

In all cases of cattle affected, the following symptoms and post-mortem findings were recorded by the veterinary surgeons who submitted the samples for analysis: evidence of abdominal pain, grinding of teeth, staggering, salivation, convulsions, blindness, coma, then death. Blindness itself in most instances appeared to be suggestive of lead poisoning having occurred, but a few samples examined in this laboratory from cases where there was a report of blindness sometimes gave negative results by chemical analysis and no mineral poisons of any description were found. It is possible that death in such cases was due to poisonous plants or bacterial infection.

Post-mortem appearances in all cases included detachable mucous membrane from the stomachs and inflammation of the abomasum.

It might be of some interest to describe briefly the physiological action said to take place in the bovine body after ingestion of lead.[1] Absorption from the stomachs in such cases is said to be slow or of the cumulative type, and the liver and kidney are the organs chiefly concerned in arresting lead salts during the period of absorption and elimination; it is possible that the absorbed lead is in the form of some complex albuminate. Like most metallic poisons, lead is eliminated slowly, by the urine and faeces, although a portion may be re-absorbed and carried back to the liver.

Prior to chemical analysis and estimation, an examination was made of all stomach contents received. In three of the cases under review, lead paint was found in the form of small particles which were seen to be black externally and white internally.

METHOD—

Reagents—*A*. Chloroform A.R. *B*. Aqueous citric acid solution, 5 g. per 100 ml. *C*. Aqueous potassium cyanide solution, 5 g. per 100 ml. *D*. Dithizone solution in chloroform, 10 mg. in 400 ml. *E*. Lead-free reagent, prepared by mixing 30 ml. of reagent *C*, 15 ml. of reagent *B* and 5 ml. of aqueous ammonia (sp.gr. 0·880) and diluting to 450 ml. with distilled water. *F*. Lead-free reagent, prepared by mixing 10 ml. of reagent *C* and 5 ml. of aqueous ammonia (sp.gr. 0·880) and diluting to 500 ml. with distilled water. If necessary, reagents *E* and *F* can be freed from lead by extraction with dithizone solution.

Procedure—Ignite 20 g. of sample in a crucible after adding 1 ml. of diluted sulphuric acid (1 : 5). Then ash at 500° C. for 2 hours. (If the sample is in liquid form add the sulphuric acid, evaporate to dryness on a water-bath and then ash at 500° C.) Cool and add 1 ml. of concentrated hydrochloric acid and place the basin on a steam-bath for several minutes; add 2 ml. of diluted nitric acid (1 : 5). Filter into a 100-ml. graduated flask, wash well with distilled water and make up to the mark; mix thoroughly. Pipette an aliquot of the solution into a 100-ml. beaker and neutralise with diluted aqueous ammonia (1 : 3). Then make acid with 5 per cent. citric acid solution and finally alkaline with ammonia. Transfer the solution from the beaker to a 250-ml. separating funnel, add 15 ml. of lead-free reagent *E* and 10 ml. of dithizone solution *D*. Shake well and allow to settle. Run off the dithizone layer into another funnel and re-extract the aqueous layer with 10 ml. of dithizone solution, or until the added dithizone solution remains green.

To the combined dithizone extracts add 20 ml. of lead-free reagent *F*. Shake well, allow to settle and repeat until the added reagent *F* remains colourless.

Run the dithizone extract into a 50-ml. flask and make up to the mark with chloroform. Match in a colorimeter against a standard solution which has been treated in exactly the same manner.

Photometric estimations can be carried out by preparing a curve from a series of standards prepared from standard lead acetate solution.

Quantitative estimations carried out by the above method gave the following results. For the purposes of comparison the figures have been converted to mg. per 100 g. of material as received.

Case No.	Subject	Rumen contents	Reticulum contents	Abomasum contents	Liver	Kidney
C.32	Heifer	1·03	56·9	10·14	4·36	16·35
C.34	Calf	8·27	11·02	149·7	7·36	28·7
C.37	Roan cow	1·65	*	*	7·18	48·2
C.38	Heifer	*	*	8·3	8·35	nil
C.123	Calf	84·0	*	35·0	*	*
C.155	Stirk	68·6	178·0	106·0	12·1	21·7
E.58	Calf	38·4	*	70·2	11·8	23·2
E.59	”	42·0	*	*	10·7	nil
E.75	Stirk	72·0	*	*	11·3	*
E.100	Heifer	*	*	89·0	10·6	25·1
E.101	”	*	*	38·7	11·2	26·3
E.103	Calf	*	*	*	9·7	*

* Denotes no sample received.

The data show that considerable amounts of lead were found in stomach contents except in No. C.38 and that the figures for kidney are much higher than those for the liver.

There is no evidence that lead is a normal constituent of the body in anything more than insignificant amounts; and the estimation of lead in liver and kidney proved those organs to be valuable material in the investigations carried out in this laboratory. It might, therefore, be safe to say that the figures given for these organs are of considerable diagnostic value, but on the other hand it is difficult to arrive at a safe and definite figure for a toxic dose.

REFERENCE

1. Lander's *Veterinary Toxicology*, 3rd Edition, 1945.

DEPARTMENT OF VETERINARY HYGIENE
ROYAL (DICK) VETERINARY COLLEGE
EDINBURGH, 9

D. J. BROWN
December, 1948

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1949, **74**, 464).

Public Analyst	*Appointments*
ALCOCK, Arthur	County Borough of Salford.
BUTTON, Donald Frank Harrington	Metropolitan Borough of Islington.
JENKINS, Daniel Ceiriog Evans	Borough of Hereford.
JONES, Daniel Evans	County Borough of Merthyr Tydfil.
WORDSWORTH, Charles Harcourt	Metropolitan Borough of Paddington.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

NOTIFICATION of the following appointment has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 464).

Official Agricultural Analyst	*Appointment*
ALCOCK, Arthur	County Borough of Salford.

Ministry of Food

STATUTORY INSTRUMENT*

1949.—No. 1656. The Food Standards (Table Jellies) Order, 1949. Price 1d.

This Order, which came into force on September 11*th,* 1949, *and which should be read with the Food Standards* (*General Provisions*) *Order, prescribes standards for Table Jelly Tablets, Table Jelly Crystals and Table Jelly Compounds.*

The Schedule to the Order sets out the standards as below :—

1. The standard for table jelly tablets shall be as follows:—
   (*a*) Table jelly tablets shall consist of sugar, gelatine or other jelly-forming material (other than farinaceous products) with other ingredients (not being farinaceous products) in tablet form. When made up with water, the tablet shall produce a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of soluble solids contained in the tablets shall be not less than 72 per cent. by weight.
   (*c*) The percentage of sugar contained in the tablets (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 63 per cent.
2. The standard for table jelly crystals shall be as follows:—
   (*a*) Table jelly crystals shall consist of sugar and gelatine or other jelly-forming material (other than farinaceous products) with other ingredients (not being farinaceous products) in crystal form. When made up with water, the crystals shall produce a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of sugar contained in the crystals (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 84 per cent.
3. The standard for table jelly compounds shall be as follows:—
   (*a*) Table jelly compounds shall consist of sugar and gelatine or other jelly-forming material with other ingredients to form a product which, when made up with milk, produces a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of sugar contained therein (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 50 per cent.
   (*c*) The percentage of starch if any contained therein shall not exceed 20 per cent. by weight.

4.—(1) "Percentage of soluble solids" means the percentage by weight of soluble solids ascertained at 20° C. by means of a refractometer on the sucrose scale, no correction being made for insoluble solids.

(2) The percentage of sugar shall be determined by adding the percentage by weight of sucrose to the percentage by weight of the total reducing sugars expressed in terms of dextrose.

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

5. The setting test referred to in paragraphs 1 to 3 of this Schedule shall be as follows:—

If the product is sold in a container with a content intended to produce one pint of a jelly table sweet, that content, or in any other case 3·75 oz. of the product, shall be made into a jelly table sweet—

(*a*) in the case of table jelly tablets or table jelly crystals by the addition of water at 88° C. approximately;

(*b*) in the case of table jelly compounds by the addition of milk at 88° C. approximately; the total volume to amount in each case to one pint.

85 mls. of the solution shall be immediately introduced into each of six beakers of approximately 5 cm. internal diameter and the beakers cooled for 18 hours in a water-bath maintained at 16° C. ± 1° C. At the end of this period the contents of the beakers shall be turned out on to a plate or dish by the following method. Each beaker shall be immersed in a water bath at approximately 50° C. for 8 seconds. Upon removal each beaker shall be immediately dried and the contents transferred to a plate or dish by inversion of the beaker. If not less than four out of the six jelly table sweets shall retain for 30 minutes the general shape of the beaker and shall not at the end of such period have collapsed or split so as to alter their shape, then the setting test shall be deemed to have been satisfied.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Preservation of Dairy Products for the Phosphatase Test. G. P. S. Sanders and O. S. Sager (*J. Dairy Sci.*, 1949, **32**, 166–174)—The following substances were tested for use as preservatives of milk intended for the Sanders-Sager phosphatase test (*Ibid.*, 1947, **30**, 909–920):—1·5 to 2 per cent. of chloroform, 3 to 3·5 per cent. of toluene, 1·5 to 2 per cent. of borax, 0·01 to 0·03 per cent. of formaldehyde, 0·05 to 0·1 per cent. of mercuric chloride, and 0·06 to 0·15 per cent. of hydrogen peroxide. All preserved fresh milk for 10 to 21 days at room temperature, but the last three produced serious chemical inhibition of the phosphatase activity and the borax interfered with the pH of the test. Chloroform was more effective than toluene as a preservative in the same concentration: neither inhibited the enzyme appreciably. Phenolic compounds should not be used as preservatives in the test because they give rise to interfering colours. The use of 1·5 to 2 per cent. of chloroform is recommended. A. H. Adams

Quick and Simple Method for Determination of Quinine and Total Alkaloids in Cinchona Bark. A. J. Loustalot and C. Pagan (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 153–159)—*Procedure*—To 2 g. of dried *Cinchona* bark ground to pass a 100-mesh sieve add about 0·5 g. of finely powdered calcium oxide and enough water (7 to 10 ml.) to make a smooth paste. After 10 min., transfer the paste to a 200-ml. flask with 100 to 150 ml. of alcohol. Shake the suspension vigorously at first and then occasionally during 1 hr., after which adjust its volume to 200 ml. and filter through a Whatman 24-cm. No. 5 paper, placing a watch glass over the funnel and a cotton wool plug in the neck of the receiving flask to minimise evaporation.

Pipette 25 ml. of the extract into a 50-ml. flask, add 25 mg. of Norit A (decolorising carbon), shake for 15 to 30 sec., and filter. To 8 ml. of the clarified extract (≡ 80 mg. of bark) add 5 ml. of 0·1 *N* hydrochloric acid, dilute to 100 ml., and determine the percentage transmittance at 380 mμ. in a photometer. Ascertain the percentage of quinine sulphate directly from a standard curve prepared as follows.

Pipette 5 ml. of each quinine sulphate stock solution (*infra*) into 100-ml. flasks, add 8 ml. of 95 per cent. alcohol, dilute to volume with water, and determine the percentage transmittance at 380 mμ., using a blank solution containing the acid and alcohol as reference solution. Plot the logarithms of percentage transmissions against the concentrations. Prepare a new curve for each set of determinations.

To determine the total alkaloids, pipette 100 ml. of the alcoholic bark extract (≡ 1 g. of bark) into a 400-ml. beaker, add 10 ml. of 0·1 *N* hydrochloric acid and 100 ml. of water, and titrate the excess of acid to pH 6·2 with 0·05 *N* sodium hydroxide and a pH meter. Make a blank determination by titrating the alcohol and acid. Subtract the value obtained with the unknown solution from the blank determination. Each millilitre of 0·05 *N* sodium hydroxide ≡ 0·0155 g. of total alkaloid.

To prepare the standard stock solutions, dissolve quinine sulphate in 0·1 *N* hydrochloric acid so that 5-ml. aliquots contain 1·6, 3·2, 4·8, 6·4, and 8·0 mg. of anhydrous quinine sulphate. This series is equivalent to 2, 4, 6, 8, and 10 per cent. of quinine sulphate when an 80-mg. sample of *Cinchona* bark is used. The solutions are stable for 6 months or more in tightly stoppered, black bottles.

At a wavelength of 380 mμ, cinchonine and cinchonidine have no effect on the absorption of light by quinine and quinidine. Solutions containing quinine alone and quinidine alone at the same concentrations give equal transmission values at 380 mμ. Since quinidine resembles quinine in its physiological effects, no serious error is incurred by its inclusion as an anti-malarial constituent. Also, quinidine is seldom present in large amount in *Cinchona* bark.

When the logarithmic percentage transmittance at 380 mμ. is plotted against concentrations of quinine sulphate ranging from 8 to 92 p.p.m., the deviation from Beer's law is small.

The method may be useful for the rapid evaluation of *Cinchona* seedlings in breeding and selection investigations and for the commercial assay of *Cinchona* bark. A. O. JONES

Determination of Nitrogen in Milk by Direct Nesslerisation of the Digested Sample. J. H. Hettrick and R. M. Whitney (*J. Dairy Sci.*, 1949, **32**, 111–112)—A rapid method for determining protein in milk by direct nesslerisation of the digested sample is described. The ammonia complex formed with coloured Nessler reagent is measured on the spectrophotometer and referred to a standard curve. The wavelength for maximum absorption is a function of the Nessler reagent used and for the most accurate work, should be determined by each investigator for the reagent he uses. The point of maximum absorption found by the authors was at 420 mμ. Small differences in the amounts of the reagents used do not affect the accuracy of the method, but the temperature must be controlled to at least $\pm 1°$ C. and the time for colour development to ± 3 min. Results obtained average 2·5 per cent. low, but are within experimental error, when compared with a macro-determination of nitrogen.

Method—Reagents—Ammonia-free water—Re-distil distilled water in a 2-litre glass still in presence of 10 ml. of concentrated sulphuric acid. Test for ammonia as below and discard if not ammonia-free. *Gum acacia solution, 2 per cent. w/v*—Dissolve 2 g. of good quality gum acacia in ammonia-free water and dilute to 100 ml. Add 1 ml. of carbon tetrachloride as preservative. This solution should be clear. *Nessler's reagent*—Dissolve 50 g. of potassium iodide in 35 ml. of cold water (ammonia-free) and add a saturated solution of mercuric chloride until the orange precipitate persists. Add 400 ml. of approximately 9 *N* sodium hydroxide solution (360 g. of sodium hydroxide in 1 litre of ammonia-free water) and dilute to 1 litre with ammonia-free water. Allow to stand several days.

Procedure—Dilute about 1 g. of milk, weighed accurately, to 500 ml. with ammonia-free water and transfer a 12·5-ml. aliquot to a micro-Kjeldahl flask. Add 0·4 g. of potassium sulphate and 0·5 ml. of concentrated sulphuric acid and digest for exactly 3 min. after the appearance of sulphur trioxide fumes. Cool and add 3 drops of 30 per cent. hydrogen peroxide directly into the liquid in the flask. Digest for exactly 3 min. more, cool to room temperature, dilute with ammonia-free water, add 1 ml. of 2 per cent. gum acacia solution, and make up to 50 ml. with ammonia-free water. Transfer a 10-ml. aliquot to a test tube and place the tubes in a thermostat at a convenient temperature $\pm 1°$ C. Add exactly 2 ml. of Nessler reagent and measure the percentage transmission on the spectrophotometer at 420 mμ. after 10 min., using a permanent colour blank. Determine the amount of nitrogen present in the aliquot from the standard curve.

Permanent colour blank—To a series of test tubes containing 10 ml. of distilled water, add 0·1, 0·2, 0·3, and 0·5 ml. of 1 per cent. potassium dichromate solution and measure the percentage transmission at 420 mμ. on the spectrophotometer, using distilled water as a blank. Plot the logarithms of the percentage transmissions against concentrations and from this curve determine the concentration of potassium dichromate necessary to yield the same percentage transmission as the average of five reagent blanks measured against distilled water. Prepare a potassium dichromate solution of this concentration to use as a permanent colour blank.

Standard curve—Transfer 0·4 g. of potassium sulphate and 0·5 ml. of concentrated sulphuric acid to each of a series of ten micro-Kjeldahl flasks containing 2, 6, 8, 10, 11, 12, 13, 14, 15, and 16 ml. of ammonia solution (1 ml. equivalent of 0·01 mg. of nitrogen) and proceed as in the method above. Plot the logarithms of the percentage transmission values obtained against the milligrams of nitrogen per 50 ml. of digested solution. A. H. ADAMS

Determination of Copper in Foods by a Two-Colour Dithizone Method. C. A. Greenleaf (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 144–152) —*Reagents*—To prepare and purify the reagents used in the method proceed as follows. Re-distil water, hydrochloric acid, nitric acid, and ammonia solution from Pyrex glass. *Carbon tetrachloride*—Shake thoroughly with 10 per cent. sodium hydroxide solution, dehydrate with anhydrous sodium sulphate, and re-distil from calcium oxide, rejecting the first portion containing moisture. *Dithizone solution*—Dissolve 30 mg. in 20 ml. of chloroform and shake out separately with a 100-ml. and a 50-ml. portion of diluted aqueous ammonia (1 : 100). Discard the chloroform, extract the ammonia solutions in series with small portions of carbon tetrachloride until the extracts are practically colourless, combine and filter the ammonia solutions, add 10 ml. of 3·2 per cent. sodium bisulphite solution (*infra*) and a slight excess of 6 *N* hydrochloric acid. Extract the dithizone with several portions of carbon tetrachloride and make it up to 1 litre. Dilute 300 ml. of this solution to 500 ml. with carbon tetrachloride. Store both solutions (designated as 25 mg. per litre and 15 mg. per litre, respectively) in a refrigerator in dark bottles under a layer of 0·1 *M* sulphurous acid (sodium bisulphite and hydrochloric acid). *Ammonium citrate solution*—Dissolve 150 g. of citric acid in 600 ml. of water, make just alkaline to cresol red, used externally, with ammonia solution, add 10 ml. in excess and dilute to 1 litre. Extract with dithizone solution in chloroform until the extracts are green, then with chloroform until the extracts are colourless and, finally, with carbon tetrachloride, and filter. *Potassium iodide solution* (10 per cent.)—To 50 g. of potassium iodide dissolved in 500 ml. of water in a separator add dithizone solution in chloroform and add ammonia solution drop by drop with shaking until the dithizone begins to enter the aqueous phase. Extract with further portions of dithizone solution until the extracts are green, then add hydrochloric acid drop by drop until the dithizone is precipitated from the aqueous phase. Extract with carbon tetrachloride and filter. If free iodine appears on standing, discharge the colour with dilute sodium thiosulphate or bisulphite solution. *Acid potassium phthalate* (0·1 *M*)—Dissolve 10·2 g. of the salt in 500 ml. of water, extract with dithizone solution in carbon tetrachloride, then with carbon tetrachloride and filter. Purify 3·2 per cent. *sodium bisulphite solution* in the same manner. *Sulphuric acid* is the least controllable reagent contributing copper to the blank determination; it usually contains 1 or 2 p.p.m.

Procedure—Prepare the sample by wet combustion with nitric and sulphuric acids. On the macro-scale take an amount of sample equivalent to 20 to 100 µg. of copper and use 20 ml. of sulphuric acid. When the digest remains yellow and does not char on evolution of sulphur trioxide add a little more nitric acid and 5 ml. of 60 to 70 per cent. perchloric acid, heat until the perchloric acid is expelled, cool, add 50 ml. of water and re-heat to fuming-point, cool, and dilute to 200 ml. With micro-digestion apparatus reduce the amounts of sample and acids proportionately and dilute the digest with 10 to 20 ml. of water before transferring to a separator.

To 10 ml. of ammonium citrate solution in a separator add the digested micro-sample or a 20-ml. aliquot of the macro-digest, dilute to about 85 ml., add 0·5 ml. of bromophenol blue indicator, and aqueous ammonia solution until the bluish tinge of the indicator appears and then *N* hydrochloric acid, drop by drop, until the colour just changes to yellow (pH 3·0 to 3·3), avoiding excess of acid. Add 10 ml. of 10 per cent. potassium iodide solution and extract with 20 ml. of dithizone solution in carbon tetrachloride, shaking vigorously for 2 or 3 min. If the extract is red add more dithizone solution and shake again. Rinse down the separator walls with carbon tetrachloride, displace floating drops of dithizone solution, draw off all but about 5 ml. of the aqueous layer by suction, rinse the stopper and walls with 25 ml. of water, but do not shake, and again remove the upper layer by suction. Add 2·5 ml. of 2 per cent. potassium iodide solution, shake vigorously and draw off the dithizone solution into a 125-ml. separator, using small amounts of carbon tetrachloride to displace floating drops and to wash dithizone solution from the tap bore and stem. Discard the aqueous layer. To the extract add 25 ml. of 0·01 *N* hydrochloric acid and saturated bromine water, drop by drop, with shaking until the dithizone layer is yellow. Shake vigorously for 1 min. to transfer the copper to the hydrochloric acid and then draw off the carbon tetrachloride layer. Wash the hydrochloric acid extract with 10 ml. of carbon tetrachloride, discard this washing, and remove floating drops and the carbon tetrachloride in the tap bore and stem by means of more carbon tetrachloride. Finally, apply suction to the top of the separator and admit a stream of air through the tap until all the carbon tetrachloride remaining in the liquid has been removed by aspiration. If the amount of copper is less than 12 µg. (as shown by absence of appreciable colour change in the preliminary extraction) use the entire solution for the estimation. If purplish hues develop draw off the copper solution, rinse the separator with 0·01 *N* hydrochloric acid and make the solution and rinsings up to a definite volume with 0·01 *N* hydrochloric acid.

To the 25 ml. of solution (or an aliquot diluted to 25 ml. with 0·01 *N* hydrochloric acid) add 5 ml. of acid potassium phthalate solution and 1 ml. of sodium bisulphite solution. From a burette add dithizone solution (15 mg. per litre) with intermittent vigorous shaking until an excess is present, as shown by a purple or greyish-purple colour. Add enough carbon tetrachloride to make exactly 15 ml. and shake vigorously for 1 min. If more than 12 ml. of dithizone solution are required make up to 30 ml. and double the amounts of the other reagents and also double the amount of copper obtained from the calibration curve. Draw off the carbon tetrachloride solution through a cotton wool plug into a test tube or small flask and determine the optical density at 520 mµ. and at 625 mµ. against carbon tetrachloride as reference liquid.

To construct the calibration curve place in a series of separators 25-ml. portions of 0·01 *N* hydrochloric acid containing respectively 0, 3, 6, 9, and 12 µg. of copper. Add the acid potassium phthalate and sodium bisulphite solutions. To the first separator add a small excess of dithizone solution as described (*supra*) and carbon tetrachloride to 15 ml. To the others add increasing amounts of dithizone solution up to the maximum amount that can be read accurately in the spectrophotometer (8 ml. at zero copper and 14 ml. at 12 µg.). Extract and determine the optical densities of the extracts at 520 mµ. and 625 mµ. as before. It is preferable to make two or three determinations of each concentration of copper by using several series of separators. From the results derive the calibration equation, preferably by the method of least squares, in the form $C = AD_{520} - BD_{625} - C_0$, where C is the concentration of copper (µg. per 15 ml.), D is the optical density at the indicated wavelength, and A, B, and C_0 are arbitrary constants. In developing the method a Coleman Universal photo-electric spectrophotometer was used with square cells of $\frac{1}{2}$-in. depth, No. ST–10–S, and the calibration equation took the form

$$C = 21{\cdot}8D_{520} - 6D_{625} - 0{\cdot}6$$

To avoid interference by the yellow oxidation product of dithizone this reagent should be prepared and stored in the manner described. Interference by bismuth, silver, and mercury is prevented by the use of potassium iodide and, although zinc and cadmium would be extracted in the preliminary stages, the acidified potassium iodide removes them entirely.

A. O. Jones

Cerimetric Determination of Small Quantities of Arsenic in Drugs after Reduction with Hypophosphite. P. Paulssen (*Pharm. Weekblad*, **1949, 84,** 33–38)—The method, as applied to arsenic pills (containing liquorice and sugar), is as follows.

Procedure—Rub down two pills (containing about 1·5 mg. of arsenic) with 10 ml. of 25 per cent. hydrochloric acid, and add 50 mg. of potassium chlorate. After 5 min., heat on the water-bath for 10 min., cool, and repeat the operation with a further 50 mg. of potassium chlorate. Filter through cotton wool, wash the filter with 20 ml. of 6 N hydrochloric acid, and add 10 ml. of 10 per cent. calcium hypophosphite solution, and a small amount of finely divided asbestos. Heat for 30 min. on the water-bath, boil for 5 min., and allow to stand until cold. Collect the precipitate on a perforated platinum plate or sintered-glass filter (1 cm. diameter) covered with a layer of asbestos about 7·5 mm. thick. Wash the precipitate with water and alcohol, and finally with water, remove the filter disc from the tube, transfer it to a 100-ml. flask, and add 25 ml. of 0·008 N ceric sulphate solution.

After the arsenic has dissolved (in about 15 min.), add a few drops of 0·25 per cent. osmic acid solution in dilute sulphuric acid and 1 drop of 0·025 M *o*-phenanthroline - ferrous solution, and titrate the excess of ceric sulphate with 0·005 N arsenious acid solution to the first change to red. One ml. of the ceric sulphate solution corresponds to 0·120 mg. of arsenic. The method can also be used for atoxyl and neo-salvarsan. G. MIDDLETON

Biochemical

Colorimetric Determination of Blood-Chloride by the Iodimetric Method. H. A. Stiff, jun. (*J. Biol. Chem.*, 1948, **172**, 695–698)—A colorimetric method that uses the same reagents as the volumetric method of Van Slyke and Hiller (*Ibid.*, 1947, **167**, 107) is given.

Procedure—Place 5 ml. of the phosphoric - tungstic acid in a 10-ml. centrifuge tube and add 0·2 ml. of serum or plasma from an accurate wash-out pipette. In another tube place 5 ml. of a mixture of 10 ml. of 0·1 M sodium chloride with 250 ml. of phosphoric - tungstic acid. Add to each tube about 60 mg. of silver iodate with a glass spoon. Stopper with a rubber stopper and shake vigorously for 40 sec. Centrifuge for 1 min. at 2500 r.p.m. To each of two Evelyn colorimeter tubes add about 200 mg. of sodium iodide and 5 ml. of water. Transfer 0·5 ml. of the clear supernatant layer from each centrifuge tube to its respective colorimeter tube. Add to each tube 10 ml. of a solution of 7·5 ml. of 0·02303 N sodium thiosulphate diluted to 200 ml. Read the absorption at 420 mμ. against a water blank.

Maximum absorption is at 400 mμ., and there is no change in the colour intensity up to a period of 5 hr. The intensity varies slightly with temperature and so a standard is necessary for each set of determinations. W. S. WISE

Colorimetric Method for the Determination of Citric Acid in Blood and Plasma. G. H. Wolcott and P. D. Boyer (*J. Biol. Chem.*, 1948, **172**, 729–736)—The method, sensitive to from 5 to 50 μg. of citric acid, is based on the well-known formation from citric acid of a brominated acetone derivative (presumably principally pentabromo-acetone), and on a new micro-colorimetric method for the determination of bromide.

Method—Reagents—(1) 1 N Sodium hypochlorite in 0·1 N sodium hydroxide. Bubble chlorine, with stirring and cooling, through a solution of 88 g. of sodium hydroxide in about 1·5 litres of water until 71 g. of chlorine have dissolved. Dilute the solution to 2 litres. The concentration of sodium hydroxide should be between 0·08 N and 0·12 N. To 1 ml. of hypochlorite reagent add 5 ml. of water and about 5 drops of 30 per cent. hydrogen peroxide. When no more oxygen is evolved on adding more peroxide, titrate the alkali, using phenol red as indicator. (2) 2 M Hydrazine. Dissolve 32·5 g. of hydrazine sulphate in 50 ml. of 5 N sodium hydroxide and dilute to 500 ml.

Procedure—Add 1·0 ml. of fresh whole blood (oxalated or heparinised) or 1·0 ml. of plasma to 4·0 ml. of 10 per cent. trichloroacetic acid contained in a centrifuge tube. Shake well and then set aside for 10 min. Centrifuge and then place 4·0 ml. of the clear supernatant layer in a 125-ml. separating funnel. Add to this 5·0 ml. of 20 N sulphuric acid and 3·0 ml. of water. Cool to room temperature.

Prepare a series of standards for a calibration curve. Place in a 125-ml. separating funnel 5·0 ml. of 20 N sulphuric acid, 4·0 ml. of 8 per cent. trichloroacetic acid solution and 3·0 ml. of a series of standard citric acid solutions containing from 5·0 to 50·0 μg. of citric acid. Prepare a reagent blank with water in place of the citric acid solution.

Add to the contents of the funnel 1 ml. of a mixture of 90 ml. of 1 M potassium bromide with 10 ml. of 1 M sodium bromate. Add slowly, with vigorous agitation, 2·0 ml. of 0·5 M potassium permanganate. Allow to stand for 10 min. and then slowly add the 2 M hydrazine solution until the solution is decolorised (about 1 ml.). Wash the sides of the funnel with 1 to 2 ml. of water. Add 15 ml. of light petroleum (b.p. above 60° C.), tightly stopper the funnel with a clean rubber stopper, and shake well for 5 min. (preferably on an automatic shaker). Release the pressure, allow the layers to separate, and then discard the aqueous phase. Wash the petroleum layer and the sides and top of the funnel four times with glass-distilled water. Transfer the extract to another separating funnel and rinse the first funnel three times with light petroleum. Wash the extract twice more with water. Remove the aqueous layer.

Add to the contents of the funnel 15 ml. of a solution, freshly prepared every 10 days, containing 0·05 M sodium sulphite and 0·40 M sodium dihydrogen phosphate. Tightly stopper the funnel with a clean rubber stopper and shake well for 5 min. Allow the layers to separate, take a 10-ml. volumetric flask, the neck of which is rinsed with a few drops of water. Heat the flask in boiling water for about 5 min. and then add exactly 2 ml. of the hypochlorite solution. Rinse the neck of the flask twice with less than 1 ml. of water, mix the contents and continue heating for exactly 4 min. from the time of the addition of the hypochlorite. Add 1·0 ml. of 6 M sodium formate, remove the flask from the bath, and shake the contents. Rotate the flask so that the hot formate solution comes into contact with the inner wall of the neck and then cool the solution to room temperature. Add 1 drop of a solution of 10 g. of ammonium molybdate per 100 ml. and rinse the sides of the flask with 5·0 ml.

of 3 M phosphoric acid. Mix the contents and add 1·0 ml. of potassium iodide solution (5 g. per 100 ml.; the solution must not be coloured yellow). Make up to the 25-ml. mark and transfer to a colorimeter tube. After 10 to 15 min. read the absorption at 400 mμ. against a reagent blank.

The Beer's law curve is not linear when a standard curve is prepared with bromide. A linear curve is obtained with citric acid standards. This is an artefact probably due to a higher yield of bromoacetone derivative with higher citric acid concentrations.

Low results were obtained in the presence of large amounts of glucose, which can be detected by an increased consumption of permanganate. In the presence of large amounts of glucose, use the modification of Goldberg and Bernheim (*Ibid.*, 1944, **156**, 33).

Acetone bodies may interfere. If samples are known to contain acetone or acetoacetic acid, take a 4·0-ml. trichloroacetic acid aliquot and boil with 10·0 ml. of 10 N sulphuric acid until the total volume is less than 10 ml. Bring the volume to 10 ml. and transfer to a separating funnel, the rinsing being done with two 1-ml. portions of water. Carry out a preliminary bromination by adding 1 ml. of a mixture of 50 ml. of 1 M potassium bromide and 10 ml. of 1 M sodium bromate. Allow to stand for 30 min. and then extract with three 10-ml. aliquots of carbon tetrachloride. Discard the carbon tetrachloride layer, which contains any free bromine or ether-soluble brominated compounds. Carry out the determination as above.

W. S. Wise

Turbidimetric Method for the Assay of Hyaluronidase. A. Dorfman and M. L. Ott (*J. Biol. Chem.*, 1948, **172**, 367–375)—Previous methods for estimating hyaluronidase have been either inaccurate or time-consuming. The following method is not open to these objections. A mixture of hyaluronic acid and acidified albumin produces a turbidity that is directly proportional to the hyaluronic acid content up to 2·0 mg. Above this, a clot forms. The turbidity development shows a maximum at pH 3·82, is sensitive to the ionic strength of the solution, and decreases with time. By using standard conditions, the turbidity after treatment of the solution for a fixed time with hyaluronidase is directly proportional to the enzyme concentration.

Materials—(1) *Hyaluronic acid*—Wash human umbilical cords free from blood and store in cold acetone. Prepare an "acetone powder" by grinding in a meat grinder and washing with acetone. Stir vigorously together for 2 hr. 200 g. of dry powder, 1200 ml. of Hayem's solution, and 3000 ml. of water. Centrifuge and then filter through glass wool. Precipitate the hyaluronic acid by pouring the solution into 12 litres of cold acetone. Wash three times with cold acetone, filter, and wash twice with alcohol and anhydrous ether. Dry over phosphorus pentoxide for 24 hr. This procedure gives a yield of 6 per cent. and the product has a relative viscosity of 3·0 at a concentration of 10 mg. per ml. For the determination, use a solution containing 3·0 mg. per ml. in 0·3 M phosphate buffer of pH 5·5. The solution so prepared is slightly opalescent, but can be obtained water-clear by filtration through a Seitz filter. Dilute to a standard turbidity—this usually requires a 2 : 3 dilution. (2) *Acidified horse serum albumin*—Prepare crystalline horse serum albumin (Kekwick, *Biochem. J.*, 1938, **32**, 553). Dissolve 1 g. of the purified albumin in 1 litre of 0·1 M acetate buffer of pH 4·1 and then adjust the pH to 3·75 with 4 N hydrochloric acid. This solution can be stored indefinitely at 4° C.

Procedure—Make up the enzyme solution in 0·5 ml. of 0·2 M borate buffer of pH 7·5 mixed with 0·5 ml. of 0·9 per cent. saline. Add 1 ml. of hyaluronic acid solution. Keep at 38° C. for 45 min. in cuvettes. Then add 10 ml. of acidified albumin reagent at room temperature and shake well. After exactly 5 min., read the absorption at 600 mμ.

W. S. Wise

Agricultural

Determination of the Lignin Content of Fresh Plant Tissue without Preliminary Drying. D. MacDougall and W. A. DeLong (*Canadian J. Res.*, 1948 [*B*], **26**, 468–471)—There is some evidence that the drying of succulent plant tissue at high temperatures either causes combination of lignin with nitrogenous substances or, if such complexes are previously formed, renders them more resistant to subsequent decomposition. To avoid errors due to this cause, removal of the interfering matter before drying was attempted; water saturated with ether was found to be the most effective non-acid extractant.

Procedure—Weigh out a 20-g. sample and a 75-g. sample and cut these into $\frac{1}{4}$- to $\frac{1}{2}$-in. pieces. Dry the 20-g. sample at 105° C. and use the 75-g. sample for lignin determination. Place the sample in a Waring Blendor with 450 ml. of water, add 50 ml. of ether and, after blending for 30 min., separate the residual material by centrifuging and repeat the extraction twice. Reflux the residue with 1 per cent. hydrochloric acid solution for 3 hr., using 150 ml. for each gram of dry matter in the original sample. Estimate the volume at this point. Filter, air-dry the material at room temperature, and extract it in a Soxhlet extractor with ethanol-benzene (1 + 2) for 30 hr. and use the final residue for lignin determination.

This method was studied with oat plants of various stages of growth and was compared with the standard A.O.A.C. extraction procedure (*Methods of Analysis*, 5th Ed., 1940). Lignin was determined by the method of Manning and DeLong (*Sci. Agr.*, 1941, **22**, 69) and the lignin for nitrogen and methoxyl determinations was isolated by filtering in sintered-glass crucibles with naphthalene as filter aid (Mueller and Herrmann, *Tech.-Wiss.*, *Teil, Papierfabr.*, 1926, **24**, 185; *Chem. Abst.*, 1926, **20**, 2746). Nitrogen was determined by the micro-Kjeldahl method with the digestion mixture of Campbell and Hanna (*J. Biol. Chem.*, 1937, **1**, 119). For methoxyl determinations by the Viebock and Schwappach modification of the Zeisel method, as described by Clark (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 136; *Analyst*, 1932, **57**, 402), samples were weighed in gelatin capsules and a suspension of red phosphorus was used in the scrubber, as recommended by Samsel and McHard (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 750; *Analyst*, 1943, **68**, 27). The hydriodic acid used was prepared as described by Clark (*loc. cit.*).

The results obtained showed that the modified procedure gives a lower apparent lignin content for young succulent tissues than the standard method. The difference decreases as the age of the tissue from which the lignin is extracted increases. Lignin isolated by the modified procedure always contains less nitrogen than that isolated by the standard procedure, and it is thus apparent that interference by nitrogenous bodies has been lessened. The absolute amount of methoxyl isolated by the standard method is greater than that isolated by the modified procedure, except with mature tissue, and thus there is apparently less interference by methoxy-containing carbohydrates in the modified procedure.

Results with field-grown oat plants show that about 50 per cent. of the difference in the results obtained by the two methods is due to modification of the pre-treatment of the sample and not to the drying of the material for the standard method, the main difference being that the acid treatment is preceded in the modified method by cold ether-water extraction and in the other by hot water extraction. With young tissue, results obtained by the modified procedure are more trustworthy than those obtained by the standard method, but with mature tissue, in which the amount of interfering matter is comparatively low, the standard method appears to give trustworthy results.

A. O. JONES

Organic

Colorimetric Determination of Small Quantities of Formaldehyde by means of Chromotropic Acid. T. Kleinert and E. Srepel (*Mikrochem.*, 1948, **33**, 328–332)—Formaldehyde yields a violet-red colour when warmed with chromotropic acid in 72 per cent. sulphuric acid. The reaction is selective for formaldehyde in the presence of many other aldehydes, *e.g.*, acetaldehyde, propionaldehyde, *n*- and *iso*-butyraldehyde, *iso*-valeraldehyde, oenanthol, crotonaldehyde, chloral hydrate, glyoxal, aromatic aldehydes, and furfural. The foreign aldehyde may be in considerable excess. The qualitative reaction described by Eegrive (*Z. anal. Chem.*, 1937, **110**, 22) shows considerable variations in colour intensity, and various factors are investigated to render the method suitable for quantitative work. The resulting procedure is suitable for 0·002 to 0·05 mg. of formaldehyde, and the error varies from 2 to 5 per cent. when the Lange colorimeter is used.

Procedure—Heat 90 ml. of 76 per cent. sulphuric acid to 60° C. in a tall 150-ml. beaker. Add 5 ml. of a freshly prepared 1 per cent. solution of chromotropic acid in 72 per cent. sulphuric acid and then, with stirring, 5 ml. of the solution for test. Keep at 60° C. in a water-bath for 20 min. with frequent stirring, then cool quickly in cold water and allow any air bubbles to disperse. Determine the formaldehyde content from the colour intensity, a colorimeter with comparison standards containing known amounts of formaldehyde being used.

W. C. JOHNSON

Practical Test for Estimating Storage Stability of Gasolines. E. L. Walters, D. L. Yabroff, H. B. Minor, and H. E. Sipple (*Anal. Chem.*, 1947, **19**, 987–991)—A modification of the extrapolated gum-time method (Walters *et al.*, *Ind. Eng. Chem.*, to be published) is presented, in which the accelerating effect of oxygen pressure is eliminated and the effect of temperature is moderated to the extent that a single gum-time or ageing gum determination gives a trustworthy prediction of storage stability, which is as accurate as the longer method and yet is insensitive to the effect of experimental error.

The effect of temperature on gasoline stability at constant oxygen pressure can be expressed by the equation $\log(t_1/t_2) = B(1/T_1 - 1/T_2)$, where t_1 and t_2 are the gum-times at the respective absolute temperatures T_1 and T_2, and B is the temperature coefficient. The oxygen pressure effect at constant temperature is expressed by $\log(t_1/t_2) = D\log(P_1/P_2)$, where t_1 and t_2 are the gum-times at the respective absolute oxygen pressures P_1 and P_2 (lb. per sq. in.), and D is the oxygen pressure coefficient. The value of B for different gasolines ranges from about 5000 to about 6000, averaging about 5500. Temperature extrapolations are normally made from 100° C. (test temperature) to, say, 90° F. (storage temperature). Assuming a B value of 5500, extrapolations for this temperature range could be in error by a factor of about 2 if B were actually 500 units different from the assumed value. This possible uncertainty is greater than desired, but could be reduced appreciably by reduction of test temperature, although at the expense of increased test time. Another uncertainty is the effect of oxygen pressure, which is small for some gasolines and rather large for others. Oxygen pressure can be eliminated as a test accelerant, especially at lower temperatures (Yabroff and Walters, *Ind. Eng. Chem.*, 1940, **32**, 83), by conducting the ageing test at atmospheric pressure, provided a sufficiently high air-to-gasoline volume ratio is maintained to avoid oxygen depletion effects (Walters *et al.*, *loc. cit.*).

Accordingly, the simplified procedure comprises oxidation of gasoline under 1 atmosphere of air at some temperature below 100° C., and the results are then extrapolated to the desired storage temperature by assuming a value for B of 5500. The uncertainty in the extrapolation arises primarily from the assumption of an average value for B and not from exponential accentuation of experimental errors, as in the complete extrapolated gum-time procedure.

The uncertainty factor (UF), caused by the use of an average value for B, can be defined by $\log(UF) = \Delta B(1/T_{ex} - 1/T_{ac})$, where T_{ex} and T_{ac} are the absolute temperatures of extrapolation and of the accelerated test respectively, and ΔB is the positive difference between the assumed and actual B values. At a given temperature, the true storage life is equal to the extrapolated storage life multiplied or divided by the uncertainty factor UF, according as the assumed B value is larger or smaller than the true one. The extent of extrapolation or magnification factor (MF) can be expressed as the ratio of the times (in months) at the extrapolated temperatures to those (in days) at the accelerated temperatures. MF can then be defined by $\log(MF) = B(1/T_{ex} - 1/T_{ac}) - 1{\cdot}4829$. The predicted storage times or temperatures can be conveniently related to the accelerated test measurement by means of a nomograph constructed from the equation defining MF (*supra*). Thus a single ageing time of 24 hr. at 69·9° C. would be equivalent to 3 months storage at 90° F. and a 5-mg. gum-time of 3 days at 73° C. would be equivalent to 6·3 months at 100° F.

Method—The bombs should be of low-carbon, cadmium-plated steel, built to withstand a pressure of 50 lb. per sq. in. at test temperatures. No pressure gauges or valves are required because the bombs are opened and closed at atmospheric pressure. The bombs are fitted with pint, quart, or half-gallon bottles according to size. Fill the clean bottle with the desired amount of gasoline, pre-cooled to about 5° C., fit a loose glass cap over the neck, and immediately place and seal the bottle in the bomb, avoiding undue agitation of the liquid. Pre-heat the sealed bomb in boiling water to the temperature of oxidation chosen, *viz.*, for 50° C., 9 min.; 55° C., 11 min.; 60° C., 30 min.; 65° C., 15 min.; 70° C., 18 min.; 75° C., 20 min.; 80° C., 23 min. The time of testing is that between placing the bomb in the pre-heating bath before, and in the ice-bath after, the oxidation. Place the bomb in a thermostat at the prescribed temperature, allow the oxidation to proceed for the prescribed interval, then plunge the bomb into ice-water for at least 15 min. Open the bomb and remove the sample. If only a portion of the sample is to be used, chill the remainder, gently purge it with air, replace it in the re-cleaned bomb, and, repeat the heating, operating, and cooling procedure as many times as will permit the determination of gum-formation as a function of the time.

Comparative complete accelerated stability tests were made with two full-range cracked gasolines by the modified gum-time and the extrapolated gum-time methods. Agreement between the measured and predicted figures by the two methods is good.

The air-to-gasoline volume ratio in the bomb need not be controlled closely provided it is between about 3 and 25. Bomb size is not an important factor. Specific points requiring the closest attention are the use of clean, dry apparatus and bomb equipment free from leaks. If leaks occur, the apparent stability of the gasoline will increase owing to oxygen depletion effects. For this reason the use of a small positive air pressure instead of atmospheric pressure during the ageing test is advantageous. The small accelerating effect of this can be neglected in stability predictions.

A. O. JONES

Micro-analytical Detection of Glycerol with 2:7-Dihydroxynaphthalene. K. Fürst (*Mikrochem.*, 1948, **34**, 25–29)—In the direct method sulphuric acid converts the glycerol to acrolein, which then reacts with 2 : 7-dihydroxynaphthalene in the same solution to give an intensely coloured fluorescent product.

METHOD—*Reagent solution*—A solution of 0·01 g. of 2 : 7-dihydroxynaphthalene in 100 ml. of concentrated sulphuric acid. The solution, when freshly prepared, has a deep yellow colour and a green fluorescence, both of which disappear on standing overnight or on warming for a short time. Red and violet colours are produced by oxidising agents and by atmospheric oxidation. Pale red solutions may be used if a blank test is used for comparison.

Direct procedure—Add 1 or 2 drops of the solution under test to 2 ml. of the reagent solution, and heat in boiling water for 20 to 25 min. A yellow or reddish-yellow colour is obtained, according to the amount of glycerol present, and a deep green fluorescence. The colour and fluorescence are stable to long-continued heating. Sensitivity 1·5 μg.; dilution limit 1 in 33,000.

Normal monohydric alcohols, free from aldehydes, do not interfere. Glycol gives a green colour and a green fluorescence, both becoming less distinct with continued heating in the water-bath (sensitivity about 400 μg.). Sorbitol, mannitol, and dulcitol give a brownish-yellow colour with a green fluorescence (sensitivity about 200 μg.). Most mono- and di-saccharides give darkening due to carbonisation. Formic, acetic, succinic, citric, benzoic, and salicylic acids give no reaction. Lactic and malic acids give yellow colours with pale fluorescence. Some aldehydes also give colour reactions accompanied sometimes by fluorescence. When interfering substances are present the following modification of the direct procedure is prescribed—Evaporate the sample solution to dryness and evaporate the residue to dryness several times on the water-bath with 2 g. of 40 per cent. lime cream. Extract with a mixture of equal parts of alcohol and ether. Evaporate the extract on the water-bath, take up the residue in the same mixture of (anhydrous) alcohol and ether, and filter. Evaporate the filtrate to dryness in a test tube, add 2 ml. of reagent solution to the residue, and heat in boiling water for 20 min. A yellow colour with green fluorescence shows the presence of glycerol. The use of phosphoric acid in place of sulphuric acid, to avoid carbonisation of certain impurities, is not successful.

2 : 7-Dihydroxynaphthalene gives colour reactions with certain aldehydes, and this affords a means of detecting glycerol through its oxidation products.

Indirect procedure—(i) Heat 0·1 ml. of the solution for test with 0·1 ml. of 0·1 N potassium permanganate in boiling water. After 10 min. decolorise with 0·1 ml. of oxalic - sulphuric acid solution (5 g. of oxalic acid dissolved in 50 ml. of water and mixed, while cooling, with 50 ml. of concentrated sulphuric acid). Cool, and mix a few drops with 2 ml. of the reagent solution. After prolonged heating in boiling water, a red-violet to red colour indicates the presence of glycerol. Sensitivity 10 μg.; dilution limit 1 in 10,000. (ii) Mix 0·1 ml. of the solution with 0·9 ml. of fresh 3 per cent. bromine water and heat for 10 min. in boiling water. After boiling off the excess of bromine, mix 0·1 ml. of the solution with 2 ml. of the reagent solution and heat for 20 min. in the water-bath. A red-violet colour indicates glycerol.

W. C. Johnson

Potentiometric Titrations with Potassium Iodate. Part VII. Potentiometric Determination of 2-Ascorbic Acid. G. Spacu and P. Spacu (*Z. anal. Chem.*, 1948, **128**, 233–238)—The method depends on the oxidation of ascorbic acid to dehydroascorbic acid with an excess of iodine and titration of the residual iodine with sodium thiosulphate.

Procedure—To a solution containing approximately 50 mg. of ascorbic acid add a known volume of 0·1 *M* potassium iodate, 1 to 2 g. of potassium odide, and 1 to 5 ml. of 2 *N* sulphuric acid. Titrate the excess of iodine with 0·1 *M* sodium thiosulphate, a potentiometric end-point being used as above.

The results obtained with pure ascorbic acid and with vitamin C tablets are satisfactory.

J. G. Waller

Potentiometric Titration in Two-phase Systems. I. Hedlund and M. Steninger (*Acta Chem. Scandinavica*, 1948, **2**, 583–591)—The potentiometric titrations of two weak acids in the presence of each other is possible if the pK values for the two acids are sufficiently separated; if this is not so, only one end-point will be detected. If the titration is carried out in the presence of an organic solvent that is immiscible with water, then if the distribution coefficients of the acids between water and the organic solvent are significantly different, two inflections in the titration curve will appear and the two acids can be determined.

Two-phase titrations of this sort have been carried out on humulon and lupulon, two acids present in hops, light petroleum being used as the organic solvent, and the method has been employed for the separation of these acids.

J. G. Waller

Inorganic

Spectrochemical Determination of Beryllium. J. Cholak and D. M. Hubbard (*Anal. Chem.*, 1948, **20**, 970–972)—The paper describes a method, for determining beryllium in biological materials, which has been developed from that described previously by the same authors (*Ibid.*, 1948, **20**, 73; *Analyst*, 1949, **74**, 269). In this application the solution is placed in the crater of a graphite rod and dried before being made the cathode of a 10-amp. D.C. arc. The optical system is adjusted so that the light from the sensitive "cathode layer" of the arc enters the spectrograph slit and the high sensitivity provided by this method of working enables quantitative determinations to be made down to 0·001 μg. of beryllium in the arc. In the quantitative method, a stepped sector is employed, but if this is dispensed with as little as 0·00025 μg. of beryllium can be detected. Working graphs may be prepared in two ways: (1) by the convential method of "log I ratios," in which case a background correction is applied, and (2) by using the separation of the blackening curves. For satisfactory results the operating conditions must be strictly standardised, for example, the same portion of the cathode layer must be photographed each time, and therefore the arc-gap width and the optical alignment are critical. Details are given of the buffer solutions employed and their use is described at some length.

H. R. Clayton

Buffer for the pH range 5 to 7. G. Smits (*Biochim. Biophys. Acta*, 1947, **1**, 280)—Mixtures of di- and mono-sodium maleates give buffer solutions having the following advantages: no precipitation with magnesium, manganese, copper, cobalt or zinc ions, no action on aneurine or its pyrophosphate, and no effect on the carboxylase activity of yeast. The following solutions are prepared. Solution I, mono-sodium maleate: 1·160 g. of maleic acid in 0·100 *N* sodium hydroxide to 100 ml. Solution II, di-sodium maleate: 1·160 g. of maleic acid in 0·200 *N* sodium hydroxide to 100 ml.

Ml of soln. I	Ml. of soln. II	pH
0	10·0	7·76
1·0	9·0	6·82
2·0	8·0	6·49
3·0	7·0	6·26
4·0	6·0	6·08
5·0	5·0	5·93
6·0	4·0	5·79
7·0	3·0	5·63
8·0	2·0	5·36
9·0	1·0	4·99
10·0	0	3·81

G. Middleton

Effect of Melting Conditions on the Spectrographic Determination of Copper in Lead Alloys. L. C. Bannister and R. H. Price (*J. Inst. Metals*, 1948, **75**, 151–162)—In the method described for preparing lead - copper alloy standards for spectrographic analysis, the constituent metals are accurately weighed, and are then melted under conditions that reduce the formation of dross. The lead is melted in an iron ladle and is then heated from the surface by a Bunsen flame; when a two-cone, non-luminous flame is used the effect of this treatment is to reduce the dross formed in the preliminary melting. When all the dross is reduced, a weighed amount of copper is added, and heating is continued until dissolution is complete. After stirring, the alloy is cast into samples for spectrographic analysis.

In describing tests on standards prepared in this way, the authors give calibration graphs showing the effect of antimony on the calibration for lead - tin - copper alloys. Experiments carried out to determine the optimum method of sampling indicate that surface heating is desirable in re-melting lead alloys, and that there is no difference in the behaviour of samples cast as slabs, and those cast as sticks and subsequently rolled into slabs.

H. R. Clayton

Polarographic Analysis of Hydrogen Peroxide. P. A. Giguère and J. B. Jaillet (*Canad. J. Res.*, 1948 [*B*], **26**, 767–772)—The polarographic determination of hydrogen peroxide can be carried out by making use of the reduction step at −1·0 v. *versus* the saturated calomel electrode. The method is limited to peroxide concentrations of less than 0·15 per cent., owing to the oxidation of mercury by hydrogen peroxide at high concentrations. If a stationary platinum micro-electrode is used in place of the dropping mercury electrode, current-voltage curves in the range from 0 to −0·6 v. *versus* the saturated calomel electrode show a reduction step whose height is proportional to the hydrogen peroxide concentration over a much wider range, and determinations can be carried out simply by measuring the limiting current at −0·6 v. The upper limit of peroxide concentration that can be measured depends on the concentration of the supporting electrolyte, and if saturated potassium chloride solution is used, concentrations up to 0·9 per cent. can be determined.

J. G. Waller

Analytical Investigation of the Univalent Thallium Ion. R. Ripan and E. Popper (*Z. anal. Chem.*, 1948, **128**, 239–241)—The method described depends on the fact that thallous ions, in presence of calcium and ferricyanide ions, form a compound, $CaTl_2[Fe(CN)_6]$, on addition of ferrocyanide ions. If a solution containing thallous, calcium, and ferricyanide ions is titrated with a solution of potassium ferrocyanide, the end-point can be detected by the usual potentiometric method.

Procedure—To 20 ml. of a 0·05 to 0·10 *M* solution of a thallous salt add an equal volume of an approximately 0·1 *M* solution of calcium nitrate and 1 ml. of a 1 per cent. solution of potassium ferricyanide. Place a platinum indicator electrode and a reference electrode in the solution and titrate with 0·1 *M* potassium ferrocyanide.

The results are in fair agreement with known values.

J. G. Waller

Thioacetamide in Place of Gaseous Hydrogen Sulphide for Precipitation of Insoluble Sulphides. H. H. Barber and E. Grzeskowiak (*Anal. Chem.*, 1949, **21**, 192)—The reagent is easily obtained, and its odour is not offensive. Its aqueous solution keeps well. Only a small excess is necessary to cause complete precipitation and, as hydrolysis gives a relatively low sulphide ion concentration, coagulation and filtration are more rapid than when gaseous hydrogen sulphide is used. Cost per unit precipitation is also less.

M. E. Dalziel

Conductometric Titration for the Rapid Determination of Sulphur in Organic and Inorganic Compounds. A. Schöberl (*Z. anal Chem.*, 1948, **128**, 210–215)—The method involves the conversion of the sulphur to sulphate and the subsequent conductometric titration of the sulphate with barium acetate solution.

Procedure for organic compounds—Heat the sample to 700° C. in a quartz tube in a stream of oxygen, and absorb the oxides of sulphur in a solution of hydrogen peroxide. When the combustion is complete, neutralise the solution with 2 *N* aqueous ammonia, add a small amount of manganese dioxide, and heat the mixture on a water-bath to destroy any hydrogen peroxide still present. Transfer the solution to a conductivity cell and titrate with 0·05 *N* barium acetate.

Sulphur determinations on a number of organic compounds give results that are in good agreement with the theoretical values. The method can be used for many naturally occurring materials.

J. G. Waller

Fractional Titration of Amalgams as a Method of Analysis of Easily Fusible Metals. V. A. Tsimmergakl and R. S. Khaymovich (*Zavod. Lab.*, 1948, **14**, 1289–1300)—If a mixed amalgam of a number of metals can be prepared, *e.g.*, by dissolving an alloy directly in mercury, or by electrolysis of an aqueous solution of the metals with a mercury cathode, the metals can be individually extracted by shaking the amalgam with mercuric chloride solution or other oxidising agent, preferably at 100° C., the completion of extraction of each metal being shown by a sudden change in the potential of the amalgam. The individual extracts may then be used for the determinations of the single metals. Alternatively, if an accuracy to within about 1 per cent. is sufficient the volume of mercuric chloride solution expended in each extraction may be used as a measure of the content of the metal.

The process of extraction has been considered theoretically and the mathematical basis is worked out. Even with very small amounts of one metal in presence of very large amounts of another the separation can theoretically be more complete than is attained in practice by normal chemical means provided that the amalgam potentials differ sufficiently. With similar initial concentrations 0·1 per cent. accuracy should be possible with bivalent metals if the difference is 0·22 v. or more.

In practice, direct titration is less accurate than this because of the ease of oxidation of the amalgams by air, and the slowness of attainment of equilibrium. These errors are reduced and other advantages appear if the extraction-titration is carried out at 100° C. The vapour of the boiling solution displaces the air, the concentration of metals in the amalgam is increased at the higher temperature and hence the volume of the amalgam may be reduced and the boiling produces agitation of the solution and of the amalgam and thus reduces the time for the titration.

At 100° C. the solubilities of the metals in mercury are zinc 7 per cent., cadmium 21 per cent., tin 19 per cent., lead 16 per cent., and bismuth 26 per cent. The mean values of the potentials at 100° C., *versus* the normal calomel electrode have been found for the first time.

	Potential (volts)				
Medium	Zn	Cd	Sn	Pb	Bi
N KCl	1·02	0·65	0·59	0·48	0·13
N KOH	1·42	0·88	1·14	0·81	0·56

METHOD—*Apparatus*—A test tube, 4 cm. wide and narrower at the bottom, is fitted with a ground-glass stopper carrying a number of tubes (1) to a vertical water condenser with internal glass stirrer passing into the test tube, (2) electrolytic bridge tube to a calomel electrode, (3) tube containing platinum wire contact to the amalgam, (4) burette jet, (5) tube for insertion of solutions or of metal, and (6) tube used for platinum wire anode when the amalgam is to be formed by electrolysis in the test tube. The capacity of the test tube is sufficient for 100 ml. of solution to be electrolysed therein when necessary. Heating is carried out by immersion of the tube in a heated calcium chloride bath.

Titration is carried out with 0·1 *N* or 0·5 *N* mercuric chloride in neutral solution, 0·4 *N* mercuric acetate in *N* acetic acid, or, most often, with 0·1 *N* or 0·5 *N* mercuric salt in *N* potassium hydroxide. This solution is prepared by dissolving 2·715 g. of mercuric chloride in a solution of 7 g. of potassium iodide in 20 ml. of water and adding a solution of 11·22 g. of potassium hydroxide, followed by dilution to 200 ml.

Direct titration—The amount of mercury taken is dependent on the solubilities of the metals present in the alloy to be analysed, and the amount of sample taken depends on the composition. Place 1 to 3 ml. of mercury in the test tube, add either 5 ml. of *N* potassium chloride or 5 ml. of *N* potassium hydroxide, heat until boiling starts, introduce 0·5 to 10 g. of the alloy, stir, and continue heating until the alloy is dissolved; then connect the tube with the other apparatus, first ensuring that the burette jet is filled with the titrating solution. Set the stirrer in motion, and connect the amalgam and the calomel electrode to a galvanometer, for measuring the E.M.F., through a resistance in series. With the galvanometer needle stable, start adding small amounts of the titrant, at intervals of not less than 1 min., to the energetically boiled and stirred solution. Stop when the needle no longer tends to a stationary position after one of these additions.

With the single metals, zinc, cadmium, lead, bismuth, and tin, the results calculated on the theoretical titre of the mercury solution were accurate to 1 part in 100. With great care and 3-min. intervals between additions, the error was reduced to 0·3 parts in 100. Similar results were obtained for the determination of tin in solder, zinc and tin in zinc - tin alloys containing small amounts of aluminium, and lead in lead - bismuth alloy. Antimony interfered in the determination of tin in Babbitt metal, and aluminium in the analysis of zinc alloys. In these cases, however, the titration may be used simply for extraction purposes, the metal extracted being determined by a suitable chemical method. G. S. SMITH

Colorimetric Determination of Small Amounts of Antimony in Copper and Tin Bronzes. E. I. Nikitina (*Zavod. Lab.*, 1948, **14**, 933–935)—Small amounts of antimony in copper and bronze can be determined colorimetrically as the complex $KSbI_4$ in sulphuric acid medium in the presence of ascorbic acid, which acts as a stabiliser of the colour and also reacts with any traces of oxidising impurities that might otherwise interfere. The ascorbic acid is introduced in the potassium iodide reagent, which contains 12 g. of potassium iodide and 1 g. of ascorbic acid in 100 ml. of water. In the presence of the ascorbic acid, decomposition to free iodine does not occur.

The colour is stable for 24 hr. The acid concentration must be at least 14 to 16 per cent. in sulphuric acid.

Determination of antimony in copper—Procedure—If tin is absent add 0·02 g. of tin to the sample weight before dissolution. Dissolve 1 or 2 g. of the sample in concentrated nitric acid, evaporate to small bulk, dilute to 30 ml. with water, add 2 ml. of 10 per cent. manganous sulphate solution and 20 drops of 4 per cent. potassium permanganate solution, and boil under cover for 20 to 30 min. Filter, well wash the precipitate on the filter with hot water, then dissolve it completely in hot hydrochloric acid and a few drops of hydrogen peroxide, collecting the filtrate in the original beaker, and washing the filter well. Add sulphuric acid to the filtrate, boil to reduce the bulk by one-half, add aqueous ammonia until the odour persists, then 10 drops of the permanganate solution, boil for 20 min. and filter. Wash the precipitate with hot water containing ammonia, and dissolve it in hydrochloric acid with hydrogen peroxide. Evaporate this solution to dryness with 5 ml. of 16 per cent. sulphuric acid solution. To the residue add 5 ml. of 16 per cent. sulphuric acid solution and, if the solution is yellow, 5 ml. of nitric acid followed by evaporation to sulphur trioxide fumes. Repeat the evaporation with nitric acid if the solution does not become colourless. Transfer the solution to a colorimeter cylinder, using 16 per cent. sulphuric acid for washing and arranging that the total volume does not exceed 15 to 20 ml. Add 4 to 5 ml. of the potassium iodide reagent containing ascorbic acid, mix, and compare the colour with a standard prepared as follows. In another similar cylinder place the same volumes of acid and reagent and add to the solution from a micro-burette a standard solution of antimony in sulphuric acid until the colour matches that of the sample.

Determination in tin bronze—Procedure—Dissolve not more than 0·1 g. of sample in 5 ml. of concentrated nitric acid, dilute to 30 ml. with water, heat for 30 min., filter through a double filter, wash first with 1 per cent. nitric acid solution, then four times with cold water, once with aqueous ammonia and finally with water again. Dissolve in hydrochloric acid with hydrogen peroxide and proceed as described above.

The method is suitable for amounts of the order of 0·001 per cent. in pure copper, and of amounts up to 0·25 per cent. in tin bronze. With 0·25 per cent., the results varied between 0·23 and 0·28 per cent., and with 0·056 per cent. between 0·05 and 0·057 per cent. G. S. SMITH

Potentiometric Titration of Quadri- and Sexa-Valent Selenium and Tellurium with Chromous Ion. J. J. Lingane and L. Niedrach (*J. Amer. Chem. Soc.*, 1948, **70**, 1997–2000)—Quadrivalent selenium and tellurium can be titrated potentiometrically with chromous sulphate solution. In each reaction the reduction product is the element. The titration curves are well-defined and suitable for analytical purposes. Although the sexavalent ions are also reducible, the reactions involved are too slow for use in analytical work.

Procedure—Place the solution to be titrated in a 250-ml., three-necked, round-bottomed flask, containing a bright platinum indicator electrode, and adjust the volume of the liquid to approximately 150 ml. Connect a saturated calomel reference electrode to the solution by a salt bridge containing dilute sulphuric acid, and pass carbon dioxide or nitrogen through the solution for 10 min. to remove dissolved oxygen. After this, allow the gas to pass slowly through the flask while the solution is titrated with 0·1 *M* chromous sulphate in either 0·1 or 1·0 *N* sulphuric acid. The best results are obtained if the solution to be titrated is about 9·0 *N* with respect to hydrochloric acid and is heated to 60° to 70° C.

Under these conditions, the oxidation - reduction potential of the selenium system is about 0·3 v. more positive than that of the tellurium system, so that both elements can be determined in one titration. Using the same conditions it is possible to determine selenium in the presence of copper.

J. G. Waller

Potentiometric Titrations with Potassium Iodate. Part II. Potentiometric Determination of Thorium. Part III. Potentiometric Determination of Lanthanum. G. Spacu and P. Spacu (*Z. anal. Chem.*, 1948, **128**, 226–229, 229–231)—The determination is carried out by precipitating the thorium or lanthanum with an excess of potassium iodate and determining the excess of iodate present by a potentiometric method.

Procedure for thorium—Transfer to a 100-ml. volumetric flask 20 ml. of a solution containing approximately 50 mg. of thorium, and add a known volume of 0·02 *M* potassium iodate so that an excess of iodate is present. Allow the precipitate to settle, dilute the solution to the mark with a measured volume of the potassium iodate solution, filter through a dry paper and then transfer 10 ml. of this solution to the titration cell. Add 100 ml. of water, 1 to 2 g. of potassium iodide, and 5 ml. of 2 *N* sulphuric acid. Titrate the solution with 0·1 *M* sodium thiosulphate, using a potentiometric method to determine the end-point (W. Böttger, *Physikalische Methoden der analytischen Chemie*, Part III, 1939, p. 692).

Procedure for lanthanum—Mix 10 ml. of the solution to be examined, containing approximately 7 mg. of lanthanum, with a measured volume (50 to 60 ml.) of 0·01 *M* potassium iodate in a 100-ml. volumetric flask. Dilute the mixture to 100 ml. with ethyl alcohol, and filter. The procedure from this stage is the same as that described for thorium.

The results for standard thorium and lanthanum solutions are in good agreement with the expected values.

J. G. Waller

Determination of Soluble Molybdates and Tungstates and Analysis of their Mixtures. H. Guiter (*Chimie Analyt.*, 1948, **30**, 180–181)—*Volumetric method*—The potentiometric titration curve of an alkaline molybdate solution with acid shows two pronounced inflexions, one at pH 10·5 to 8, corresponding to the ortho-salt (M_2O,MoO_3), and the other at pH 4·5 to 2·5, corresponding to the meta-salt ($M_2O,4MoO_3$). Similar changes occur with tungstates at pH 10·5 to 8·5 and pH 5 to 2·5. If, therefore, an alkaline solution of a molybdate or a tungstate is titrated with 0·1 *N* sulphuric acid, first to the end-point of phenolphthalein and then to the end-point of methyl orange, the concentration of the solution can be calculated from the equation $x = (T\,V/V_1)(4/3)$, where x is the concentration of the solution in half g.-mol. of MoO_3 or WO_3 per litre, T the normality of the standard acid, V (ml.) the volume of standard acid consumed between the two end-points, and V_1 (ml.) the volume of the molybdate or tungstate solution. The methyl orange must be taken to a full red colour. Although the reaction is reversible, a back titration with alkali is not satisfactory because, in this direction, equilibrium is only slowly established. But the acid titration may be repeated after addition of an excess of alkali and a delay of 15 min. The method is accurate to within ±2 per cent.

Gravimetric method—On addition of a solution of a barium salt to a molybdate solution more alkaline than pH 6·6, barium molybdate of the composition $BaMoO_4$ is precipitated. Similarly, tungstate solutions of pH greater than 6·4 yield $BaWO_4$. The precipitation should be effected at the boiling-point and the filtrates should yield a blue colour with bromophenol blue. The method is accurate to within ±1 per cent.

Analysis of mixtures of molybdates and tungstates—First use the volumetric method to determine the concentration, θ, of the solution in terms of half g.-mols. of ($MoO_3 + WO_3$) per litre. If x and y represent the respective individual concentrations in the same units, then

$$\theta = x + y \qquad \text{.. .. .. ..} \qquad (1)$$

Determine the total weight (m) in g. of mixed barium salts obtained from n ml. by the gravimetric method, then

$$n(0{\cdot}1485x + 0{\cdot}1925y) = m \qquad \text{.. ..} \qquad (2)$$

From (1) and (2), $x = (0{\cdot}1925\theta - m/n)/0{\cdot}044$ and $y = (m/n - 0{\cdot}1485\theta)/0{\cdot}044$.

The precision of this method is of the order of 3 per cent.

W. C. Johnson

Useful Stop-cock Lubricant. I. E. Puddington (*Anal. Chem.*, 1949, **21**, 316)—For stop-cocks in exposed positions the lubricant must have a low vapour pressure so that re-greasing is only occasionally needed. The thickened glycerols usually recommended tend to be short-lived in frequently turned taps. A better lubricant is a well stirred dispersion of 20 to 25 per cent. by weight of silica (Santocel C, ignited overnight at 450° C.), in glycerol that has been rendered anhydrous by heating to about 70° C. for 2 hr. in a diffusion-pump vacuum; this dehydration is especially advantageous in preparing a batch for low-pressure systems.

A 6-mm. bore stop-cock so lubricated was unaffected by exposure to 6-cm. pressure of benzene vapour for 6 months.

M. E. Dalziel

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1949, **21**, 191–192)—The crystallography of pimelic acid is described under the headings given in the original paper of the series (*Ibid.*, 1948, **20**, 275; *cf. Analyst*, 1948, **73**, 579).

M. E. Dalziel

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1949, **21**, 306–307)—The crystallography of benzalaminophenol is described under the headings given in the first paper of the series (*Ibid.*, 1948, **20**, 275; *cf. Analyst*, 1948, **73**, 579).

M. E. Dalziel

Reviews

A New Notation and Enumeration System for Organic Compounds. By G. Malcolm Dyson, M.A., D.Sc., Ph.D., F.R.I.C. Second Edition. Pp. ix + 138. London: Longmans, Green & Co., Ltd. 1949. Price 10s. 6d.

The second edition of Dr. Dyson's book on his system of notation and enumeration follows two years after the first, and in the interval he has consolidated the basis of the system and developed its features, main and subsidiary. He claims that the first edition of the book was deliberately provocative and intended to stimulate discussion and criticism. It is clear that it was most successful in attaining this object. The interest of chemists was apparent as soon as the system was presented to them, and the rapid advances that have been made in the past two years are largely due to the enthusiasm emanating from America and to the collaboration the author has received from American sources.

The major developments that have occurred and that are to be found in the second edition result from the abandonment of the principle of adduction in favour of a more logical treatment of ring systems, and in the re-arrangement of symbols within operations.

When the system first appeared it was possible to give an account of all its salient features on two pages of *The Analyst.* This can no longer be done, and more rules are now needed than formerly. These rules are presented formally in this second edition, and it would appear that some knowledge of the system is assumed. It seems rather a pity that the more elementary mode of presentation of the earlier edition should have been dropped and one would welcome the re-introduction in future editions of some of the beginning of the former Chapter III.

Once the system has been mastered, however, it is seen to be really quite simple, and there is no doubt that the latest developments have added to its elegance and usefulness. The author may well find that it will not long remain merely a "proposal for an international system." K. A. Williams

The Advertising, Labelling and Composition of Food; A Report by the Ministry of Food. An account of four years' administration of the Defence (Sale of Food) Regulations, 1943, by the Food Standards and Labelling Division of the Ministry of Food. Pp. iv + 77. London: H.M. Stationery Office. 1949. Price 1s. 6d. net.

The Report is divided into seven parts, of which the first six, occupying 11 pages, contain a general account of the reasons that led to forming a Labelling Division of the Ministry of Food and describe the state of affairs at its inception, what it set out to do, what it has done and what remains to be done in the future. These parts are of interest, more particularly, to the general public.

For public analysts, manufacturers, lawyers and candidates for the Branch E Examination of the Royal Institute of Chemistry the important section is Part VII, which contains 14 appendixes and occupies 65 pages on the following subjects: the White Paper of 1943 on the labelling and advertising of foods; the Defence (Sale of Food) Regulations, 1943; the Labelling of Food Order, 1946, with its schedules; the Food Substitutes (Control) Order, 1941; Codes of Practice (these are a set of rules that have been mutually agreed upon by the Labelling Division of the Ministry of Food and the manufacturers and packers of food products for the description and composition of 21 classes of food and their varieties); Claims for Vitamin and Mineral Contents; Review of Food Standards and Proposals for Standards; Abstracts of Standards in Commodity Control Orders; Abstracts of Standards Applied by Manufacturing Licences; the Food Standards (General Provisions) Order, 1944; the Dried Egg (Control of Use) Order, 1945; the Fluorine in Food Order, 1947; the Mineral Oil in Food Order, 1949; finally, summarised lists giving the sources of applications for the Minister's consent to proceedings under the Defence (Sale of Food) Regulations, 1943, for the years 1944 to 1948, with the results of the applications and of any proceedings.

These 65 pages of appendixes are fully documented with the numbers and dates of the relative Statutory Rules and Orders and Statutory Instruments. If by frequent revision it should be possible to keep them up to date, this Report will undoubtedly come to be regarded as a textbook on the application of Sections 6 and 8 of the Food and Drugs Act, 1938.

So far as can be seen without an extended working knowledge of the text, the important sections that deal with Statutory Orders and Instruments are accurate and complete; but an omission on page 35 requires attention: there is, on that page, no textual indication to correspond with the footnote marked "(a) S.R. & O., 1946, No. 945." This refers to the Soft Drinks Order, and should, presumably, if the arrangement of the footnotes is intended to be in their order of incidence in the text, be the last and not the first item in a list of six.

All those who are under the necessity of acquiring an accurate and complete knowledge of this particular section of the laws and regulations relating to food, with all their concomitant complications of amendments, revocations and re-enactments, will be grateful to the anonymous compilers of this invaluable compendium.

F. L. Okell

THE ANALYST

INTERNATIONAL CONGRESS ON ANALYTICAL CHEMISTRY IN 1952

THE plans for the holding of an International Congress on Analytical Chemistry in Britain in 1952 have been carried a stage further since the earlier announcement of August this year.

An approach has been made to the Council of the International Union of Pure and Applied Chemistry for its patronage.

At the meeting of the International Union in Amsterdam in September, not only was this patronage given but also the proposal was thought to be in accord with one of the major decisions taken at that meeting, *viz.*, the formation of six autonomous sections, one of which would be concerned with Analytical Chemistry.

Although these plans of the Union are tentative and will be submitted for final approval in 1951 in Washington, it is likely that a meeting of the Board of this provisionally constituted section on Analytical Chemistry could be arranged to coincide with the Analytical Congress in Britain in 1952.

The Executive Committee, under the Chairmanship of the President of the Society of Public Analysts and Other Analytical Chemists, Mr. G. Taylor, O.B.E., F.R.I.C., has had preliminary discussions with regard to location of the Congress, publication of the papers, and other matters, and a Sub-Committee is studying the scope of the subjects to be discussed.

Sir Wallace Akers, C.B.E., has kindly agreed to be Chairman of the Finance Committee and the Committee is also fortunate to have had advice and help throughout from the President of the Royal Society, Sir Robert Robinson, O.M., who continues to act as Chairman of the General Committee.

The Honorary Secretary of the Congress is Mr. R. C. Chirnside, F.R.I.C., Research Laboratories of The General Electric Co., Ltd., Wembley, England.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 6.45 p.m. on Wednesday, November 2nd, 1949, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The chair was taken by the President, Mr. George Taylor, O.B.E., F.R.I.C.

The meeting was devoted to "Some Recent Advances in Water Examination," and the following contributions on this subject were made: "Introductory Remarks," by J. H. Hamence, M.Sc., Ph.D., F.R.I.C.; "Recent Advances in our knowledge of the *Coli - Aerogenes* Group," by C. B. Taylor, D.Sc.; "The Determination of Turbidity," by Roy C. Hoather, B.Sc., Ph.D., F.R.I.C.; "The Determination of Residual Chlorine," by G. U. Houghton, M.Sc., F.R.I.C.; "The Determination of Ammonia by Direct Nesslerisation," by J. E. Houlihan, B.Sc., A.R.I.C. A discussion followed.

NEW MEMBERS

Ian McPhail Varenne Adams; Harry Baines, D.Sc. (Lond.), F.R.I.C., Hon.F.R.P.S.; Peter Reginald Walter Baker, B.Sc. (Lond.), A.R.I.C.; Robert Fisher, B.Sc. (Sydney), A.A.C.I.; Thomas Proctor Gervis, B.Sc. (Lond.), A.R.I.C.; Jefferson Marshall; Joseph Ritchie, B.Sc. (Aber.); Eli Seal, M.Sc. (Wales), A.R.I.C.; Gunter Benno Seaton; Roderick Louis Stephens, B.Sc., B.Pharm., Ph.C., F.R.I.C.; Arthur Barry Timms; Abbas Jawad Towaij, Ph.C. (Baghdad).

A JOINT Meeting of the Society with the Agriculture Group of the Society of Chemical Industry and the Fertiliser Society was held at 2.30 p.m. on Tuesday, October 18th, 1949, in the Chemistry Department of the Royal College of Science, Imperial Institute Road, South Kensington, London, S.W.7. Dr. A. H. Lewis was in the chair.

The subject of the meeting was "Fertiliser Analysis, with Special Reference to Available Phosphoric Acid," and the following four speakers made contributions: Dr. E. M. Crowther, F.R.I.C.; Mr. G. Taylor, O.B.E., F.R.I.C.; Mr. W. C. Hanson; Dr. J. H. Hamence, M.Sc., Ph.D., F.R.I.C.

BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Biological Methods Group and the Society for Endocrinology was held on Thursday, October 20th, 1949, at the Medical Society of London, Chandos Street, Cavendish Square, London, W.1, at 2.15 p.m. The chair was taken by Professor J. H. Gaddum, F.R.S.

The meeting was in the form of a symposium on "Hormone Assay," and the following aspects of the subject were presented and discussed: "General Approach to Biological Assay," by Dr. H. O. Schild; "Assay of Thyroxine and Thyroidal Activity," by Dr. G. F. Somers; "Assay of Insulin," by Mr. K. L. Smith; "Assay of Gonadotropins," by Dr. J. A. Loraine; "Assay of Posterior Pituitary Extracts," by Mr. G. A. Stewart; "Clinical Methods of Assaying Oestrogens," by Dr. P. M. F. Bishop.

Recent Modifications in the Spekker Photo-electric Absorptiometer

BY R. A. C. ISBELL

(*Presented at a meeting of the Physical Methods Group on February* 22, 1949)

THE Spekker absorptiometer dates from about 1936, when Dr. Renshaw of the Laboratory of Applied Pathology and Medicine, Manchester, proposed the use of photo-electric cells for certain pathological determinations, such as the measurement of haemoglobin and counting of blood corpuscles. The research laboratories of Adam Hilger Limited tried various arrangements, both optical and electrical, and eventually produced the Spekker absorptiometer, which had two photo-cells connected in opposition and the galvanometer was used merely as a null point indicator.

The optical balancing was achieved by means of a calibrated mechanical shutter, operated by an eccentric cam, and the instrument remained in this form until the present day. The size of the cam on the original instrument was only of about ⅝-inch diameter; the drum carrying the calibration was about 2 inches in diameter, and only 90 degrees of its circumference was taken up by the engraved scale—a length of about 1·5 inches.

The instrument remained in that form with various modifications until about the beginning of the second world war, when the form of the cam was changed so that the plunger for the movable shutter was bearing against the inside of an eccentric circular cam, the diameter of which was 3½ inches. The design of this model was also modified so that the length of the scale occupied 180° of the 4¾ inch diameter drum.

These improvements in accuracy were considered necessary, as the instrument, which at first had been used entirely for determinations in pathological work and food analysis, was becoming employed for routine work requiring greater precision, such as metallurgical analysis. In this latter field the inherent errors of sampling are very much smaller than those experienced in the earlier applications, and there was therefore justification for improving the performance of the instrument.

Much of the earlier work on metallurgical analysis was carried out by E. J. Vaughan of the Bragg Laboratory, Sheffield, and is described in his monographs[1,2] on the subject.

During the war the usefulness of the Spekker instrument increased even more rapidly, not only for metallurgical analyses, but also for sundry applications for which special adaptors were designed. Among these may be included the micro-cell adaptors for pathological work, the long cell bracket for very faintly absorbing liquids, and also the fluorimeter attachment which has become standard equipment for the analysis of aneurine.

With increasing use of the instrument, the technique also improved, and the accuracy of the chemical processing of the sample prior to measurement was also subjected to a considerable amount of research.

One further improvement which had a great bearing on the increased application in metallurgical analysis was the use of the Ilford spectrum filters, which have a very narrow waveband transmission, and the Cambridge Spot galvanometer with its increased sensitivity and very much shorter period of response: this galvanometer was also very much easier to read than the existing Unipivot needle galvanometer. With these modifications the instrument enjoyed a greatly extended use, and during this time suggestions for further improvements were received from many users, notably from the Absorptiometric Panel, which met in Glasgow, and from Dr. F. W. Haywood[3] of Wild-Barfield Electric Furnaces Ltd., whose book on the Spekker photo-electric absorptiometer, written jointly with Mr. A. A. R. Wood, provides a useful reference on metallurgical applications.

As many as possible of these suggestions were incorporated without delay and without interfering with production, but the majority of the more extensive alterations were held over until such time as a complete re-design of the instrument could be considered.

After the war the demand for the instrument did not fall off, as might have been anticipated, and in order to meet pressing requirements, it was necessary to continue with the existing design longer than had been intended. It was also apparent that in re-designing the instrument it would be necessary not only to take into account the suggestions made, but also to eliminate the manufacturing difficulties, which were increasing as the number of the instruments made increased.

When the instrument was originally designed, the demand was only a fraction of what it is at present, and therefore the methods of manufacture were comparatively simple. It becomes practicable to employ mass production methods when the quantity has increased sufficiently. It was necessary, however, to maintain the precision of manufacture, especially in the shutter mechanism, which as stated above, was a circle about 3·5 inches in diameter with an eccentricity of about 0·5 inch. The circle had to be turned true to within 0·0002 inch.

Before dealing with the shutter mechanism and drum, on the new instrument, however, brief mention will be made of a few of the other modifications that had been made to the general design of the instrument. It had become apparent from much-used instruments returned for overhaul and renovation, that as the instrument had been put more into routine use, it was gradually being put into the hands of less skilled operators, and the same care was not being taken in handling as previously, with the result that liquids were often spilt over the instrument, and corrosion was occurring, especially in the cell carrier, which in some instances was completely spoiled. After some experiments with highly resistant plastic materials, Polythene was chosen as the most inert substance available for this purpose. The cell carrier was therefore moulded from this material in the form of a tray, with three compartments, and there is therefore now little danger of the corrosive liquids being spilt on the remainder of the instrument. A moulded lid is also provided to exclude stray light.

The original lamp house had been designed for manufacture in small batches, and for use mainly with the ordinary 100-w. projector lamps, which in the earlier instruments were kept alight only for the duration of the individual readings. With the new method of working, and the use of the mercury vapour lamp, which could not be turned off between readings, more heating occurred and some doubts were raised by users as to whether overheating of the lamp occurred. Although it is not necessarily agreed that this does occur, it must be admitted that the housing becomes uncomfortably hot if the instrument is used for long periods. A new cast housing with ample ventilation and suitable fins for cooling has therefore been designed, and this has been fitted with an automatically returning shutter, which can be operated readily with either hand. As the shutter is automatically returning and the closed position gives the zero reading, it has been decided to dispense with the mercury switch which, although simple, caused a disproportionate amount of trouble in testing and adjusting the instrument. Apart from the mercury being either too sluggish or too lively, it would sometimes develop contact resistance, or generate an e.m.f. sufficient to deflect the galvanometer fully off the scale in the worst cases. These faults had, of course, always become apparent during the testing of the instrument, but unfortunately such difficulties hold up quantity production. The galvanometer, therefore, is now left permanently connected to the photo-cells, and the shutter is used instead of the galvanometer switch. This has the advantage that any small dark-currents can be automatically corrected.

The adoption of narrow band gelatin filters early in the war increased the accuracy of determination; it is of course impossible for them to last as long as glass ones, but this slight inconvenience is justified by the improvement in performance. If the instrument is operated for long periods, the heat-absorbing filters themselves become hot and radiate heat which over a period will possibly shorten the life of the gelatin filters placed close. In re-designing the instrument, therefore, the gelatin filters were moved to the far side of the solution, leaving the heat-absorbing filters close to the lamp. At the same time the long-demanded sliding filter carrier was incorporated. This has two divisions, each of which is capable of holding two or three filters. The use of plastic mouldings when practicable further reduced the possibility of heat being conducted to the filters and solutions.

At this point reference may be made to a matter that must be in the minds of many users, namely the possibility of new types of filters being available in the near future. There is little hope that glass filters will be improved within a few years to give a very narrow waveband. Combinations of new glasses may be produced in the future whose composite transmission curves may be sufficiently narrow, but the light transmitted would, as far as can be seen at present, be so weak as to be insufficient to activate the photo-cells. The same remarks at present also apply to the interference type of filter, on which experiments are being made. As far as can be forecast at present, these interference filters will also require subsidiary filters to remove the other spectrum orders which are transmitted. These subsidiary filters, however, cut down the intensity of the filtered light, and it remains to be seen therefore whether this type of filter may be used. We hope that in due course the many difficulties in this field will be overcome.

The long-cell version of the Spekker absorptiometer, which was designed for the measurement of very pale liquids, such as sugar solutions and syrup, or water containing impurities, is not so well known. This model can accommodate columns of liquid up to 20 cm. in length. Owing to the divergence of the beam of light over this length of path, it is necessary to keep the cross-sectional area of the cell comparatively great to avoid internal reflections, with the result that in the existing instrument the 20-cm. cell has a capacity of about 500 ml. Fortunately this fact has not to a great extent hindered this application, as most pale liquids are available in large quantity. However, it must be admitted that it is not convenient to move a cell carriage holding two cells containing about 1 litre of liquid, and it was therefore considered desirable to reduce the capacity of the cells in the new design. This was eventually achieved by using a low-voltage lamp with a more compact filament. As the dimensions of the source are decreased, the divergence of the collimated beam decreases also; it has been found that a 12-volt filament has a suitable width. The dimensions of the beam are further reduced by introducing a stop restricting the width of the beam at the shutter. By this means the use of a 20-cm. cell with a capacity of only 100 ml. is possible with the same precision of measurement.

Dr. F. Wokes, in the following paper,[4] describes some of his work with the fluorimeter version of the instrument, and it is a pleasure to acknowledge here the very helpful criticisms and suggestions offered by him and his assistants at all times. Furthermore, the Aneurine Panel of the Analytical Methods Committee of this Society has been a constant reminder of requirements in this field, and although the earlier Spekker fluorimeter, in its application as far as a physicist may deduce, is sufficiently accurate, it is apparent that more sensitivity would be desirable for riboflavine determinations. Aneurine and riboflavine are, of course, not the only two substances that are estimated fluorimetrically, and therefore any considerable improvement in sensitivity in this field would be welcome. During the war a special modification was evolved for the estimation of Atebrin, which fluoresces only very faintly indeed. By using a photo-cell on either side of the solution cell, the sensitivity was considerably increased, perhaps as much as 3-fold. This has been extended still further by using larger photo-cells on either side, and the over-all gain on the new model is therefore about 5-fold over the former model. At the same time the cell capacity has been reduced to about 9 ml. The fluorimeter lid cannot be opened without first obscuring the photo-cells.

Similar remarks apply to the use of the adaptor for nephelometry, although it is doubtful at present whether the full sensitivity can yet be employed when the instrument is used for detecting faint turbidity, owing to the difficulty of obtaining distilled water or solvent sufficiently free from particles. The use of plugs under each adaptor which fit into sockets in the base of the instrument facilitates change of connections. For nephelometry the spot galvanometer may be used, as the mirror galvanometer is too sensitive.

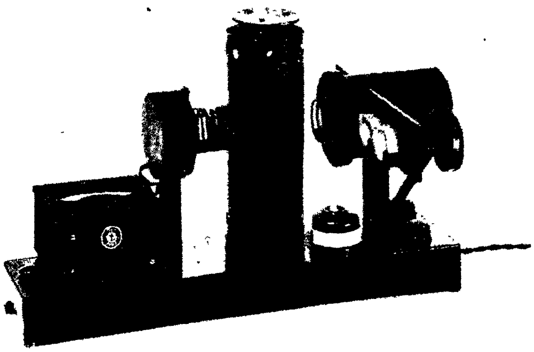

Fig. 1. The first model Spekker absorptiometer

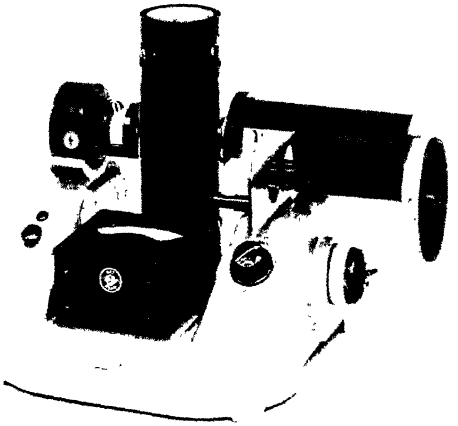

Fig. 2. An intermediate model of Spekker absorptiometer

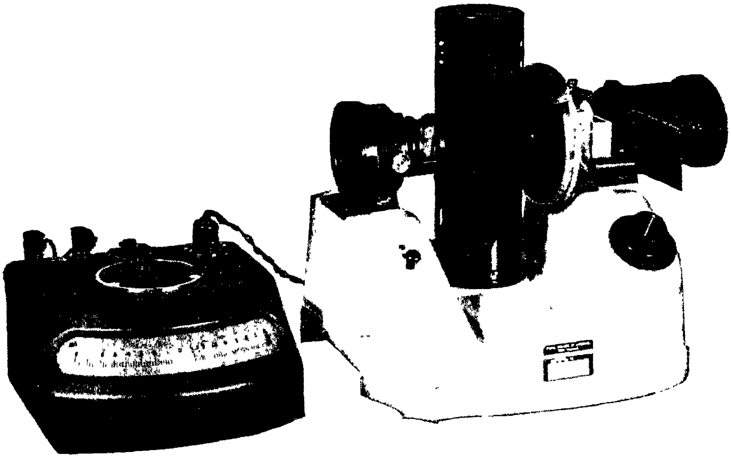

Fig. 3. The H560 model Spekker absorptiometer

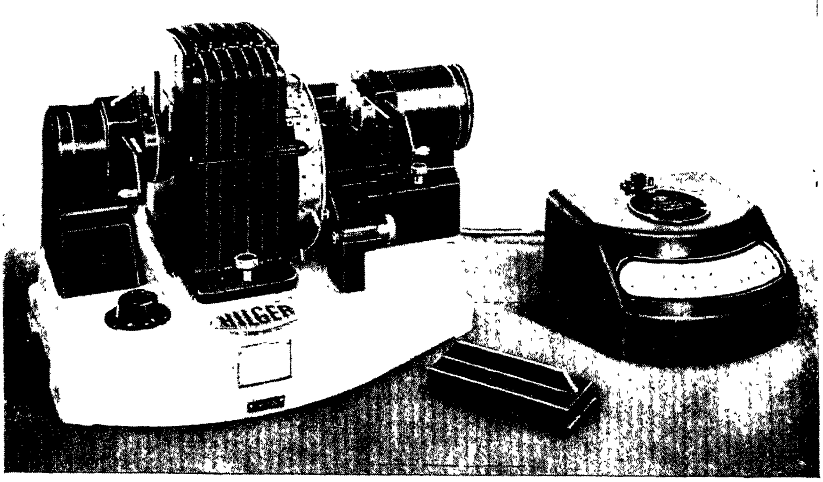

Fig. 4. The new design Spekker absorptiometer, Model H760

We now come to the greatest modification in the instrument, namely the drum shutter mechanism on which the accuracy of the instrument depends. For some years requests have been made to modify the scale on the drum so that the divisions would be uniformly spaced. It will be appreciated that the accuracy of measurement decreases as the divisions close up. It was partly for this reason that the manufacturers would not officially recommend the water-to-water 1·0 method suggested by E. J. Vaughan.[1] It is admitted that many users have adopted this method as a routine, and obtained very consistent results, but even then the manufacturers would not consider taking the responsibility of recommending this method officially, as it was felt that criticism would then be raised against the closeness of the scale at the starting point, *i.e.*, 1·0 or 1·3 as the case may be. The limit of accuracy of reproducibility of setting the drum may be taken as the thickness of the engraved line on the scale. At a reading of 1·0 this represents an uncertainty of setting ±0·003 on the blank alone. If 0 is taken as reference point, however, the divisions at this point are much more widely spaced, so that the error of the setting is negligible. Furthermore, the uncertainty caused by the closed scale occurs at high optical densities only, and therefore represents a lower percentage of the reading.

Not only does the drum reading itself introduce uncertainty, but the shutter mechanism also at higher densities is not capable of giving such a high degree of reproducibility. The height of the fully open aperture (at 0) is slightly under 0·5 inch, and therefore the height at density 1·0 is 0·05 inch, and at 1·3, 0·02 inch approximately. It will therefore be seen that if any worthwhile accuracy is to be obtained the precision required is extremely high. Thanks to very careful inspection of parts during manufacture, it has not been found difficult to maintain sufficient accuracy, and we would emphasise here what is considered to be a very important feature of the Spekker: the ultimate degree of accuracy and linearity depends mainly on mechanical precision and this can be controlled by instrument manufacturers to very fine limits. There is no dependence on calibrated potentiometers or slide wires with the added danger of varying contact resistance developing in sliding contacts. Furthermore, no reliance is placed on calibrated density wedges, each one of which would have an individual calibration. The drum units in the existing design are interchangeable. The linearity of the photo-cell response is unimportant as one compares equal intensities, and the electrical circuit is not changed during the measurement.

To revert to the main subject, however, the designers were requested by the users to produce a scale of a logarithmic function (*i.e.*, 0 to infinity) with divisions uniformly spaced. A little thought would reveal that the scale would of necessity be inordinately long whatever method we used of distorting the scale into linearity. The only solution would be to decide on a useful range of the scale, and to try to distort this into something approaching linearity. Many types of shutter were tried, most of which produced complicated equations which were insoluble, and others which were unsuitable for one reason or another. For example, in order that the micro-cell attachment should still give linear results, it was essential that the width of the beam should not be altered. Eventually this problem was solved by Mr. G. F. Lothian, who worked from the required answer, and thus produced a cam which was itself the shutter. This cam, which resembles a spiral, reduces the height of a wedge-shaped aperture as it is rotated. The scale on the drum which is associated with this is linear between 0 and 1·3, beyond which it is impossible to work without sub-standards.

The drum was now enlarged and the scale between 0 and 1·3 occupied 310° instead of 180°, making a scale length of 16 inches compared with only 6 inches on the older model for the same optical density range. It also became possible to mount the whole mechanism on precision ball-bearings, so that a perfectly free movement is obtained. The opportunity has also been taken to fit a friction driven slow motion device to improve the ease of setting.

The cam is designed so that it can be made in large numbers to within very fine limits, and there is no possibility of wear affecting the accuracy.

The possible requirements of fluorimeter users were also considered. The intensity of fluorescence bears a linear relation to the concentration, unlike absorption, which is related by a logarithmic function. A transmission or intensity scale was therefore engraved on the drum at the same time, and it is hoped that this will be of real value. This scale is superfluous for absorption measurements, but is useful where nephelometric measurements are to be made, as it is quite possible that methods may be developed for metallurgical analysis and other applications where an accurate measure of the scattering in solutions may be of use.

Finally, mention should be made of the general improvements in appearance of the instrument and the re-arrangement of controls. The electrical connections have been made as nearly foolproof as possible. Each adaptor will be fitted with a suitable plug to simplify electrical connection with the base.

It should also be noted that although each modification introduced many difficulties it has been found possible to incorporate all that was desired, and it was not eventually found necessary to limit in any way the applications or accuracy hitherto obtainable.

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DISCUSSION

Dr. W. STROSS asked the following questions:—

(*a*) Could flow-through cells also be used with the new model?

(*b*) Was it possible on the new model to exchange the ordinary bracket (allowing the use of cells up to 4 cm.) for the bracket taking 20-cm. cells just as easily as the change over from the absorptiometer to fluorimeter was performed?

(*c*) It would be of considerable practical importance if glass filters providing an equally narrow transmitted band to that of the Ilford spectrum (gelatin) filters could be obtained, and he wondered whether this was really impossible, as stated by the lecturer. According to his experience the filters supplied with the Pulfrich step-photometer were of a similar standard and *were* glass filters.

(*d*) According to his experience the "drift" of the setting was a much greater source of error than the difficulty of taking a reading in certain ranges of the scale, and he wondered whether the new model showed any improvement in respect of this drift.

(*e*) Regarding the interference filters, he thought that according to the data published[1,2] these filters had a much *greater* transmission in most ranges of the spectrum than the gelatin filters.

Mr. ISBELL replied that it is possible to use flow-through cells on the new model by replacing the standard cell-slide by a special one designed for this purpose. Furthermore, there was a special long-cell model available to take cells up to 20 cm. in length, and it was possible to convert this into a fluorimeter if necessary. Glass filters with very narrow transmission bands are not available in this country, and even if it were possible to make a combination of coloured glasses, as used on the Pulfrich photometer, such glasses were not obtainable in this country in sufficient quantity to be available for use on the Spekker absorptiometer in the numbers required. Dr. W. H. Willott of Chance Brothers, in a lecture to the Industrial Spectroscopy Group of the Institute of Physics on November 5th, 1948, explained that it was not possible to make glasses with band width comparable with those of the Ilford spectrum gelatin filters. With respect to drift in the setting of the instrument, it was possible that this would be slightly better with the new model, as a reading could be taken more quickly, and as the gelatin filter was further away from the heat-absorbing filter the former would not therefore get so hot. As explained above, although the peak transmission of interference filters was high, the total transmission was low, and thus the energy incident on the photo-cell was insufficient for satisfactory operation of the instrument.

Dr. J. E. PAGE asked whether organic solvents, such as chloroform, would affect the Polythene cell-holder. Could either a 2- or 4-cm. micro-cell be supplied? A longer micro-cell would be of considerable value in biological work. In view of the much greater sensitivity of photo-multipliers, he asked if commercial fluorimeters incorporating photo-multipliers were likely to become available in the next four years.

Mr. ISBELL replied that a sample of Polythene had been tried with various solvents and found to be quite satisfactory. According to the details supplied by the manufacturer, Polythene withstood all solvents except hot toluene. The design of a 2- or 4-cm. micro-cell would be difficult, but an experimental 2-cm. cell had recently been made, and it was hoped that it might be possible to standardise it. As to the relative merits of barrier-layer photo-cells and photo-multipliers in fluorimeters, it was the manufacturers' intention to keep the instrument as simple as possible, and it was considered that a standard fluorimeter, with the increased sensitivity provided by two large barrier-layer photo-cells, would be good enough for routine work. The modification with photo-multipliers would be more useful for exploratory or research work.

Mr. A. C. MASON suggested that the design of longer micro-cells might be simplified by using a compact source lamp, such as a Pointolite. He also remarked that in his opinion drift appeared to be connected partly with high mains voltage fluctuations.

Mr. ISBELL replied that the width of the Pointolite ball was about 2 mm., which was very little less than the present width of the filament of the lamp used with the micro-method. With regard to drift, the compensation of the instrument should be sufficient to counteract standard voltage variations, but where the voltage change was rapid and very large it was possible that the quality of the light transmitted by the filters at the end of the spectrum might be changed slightly.

Mr. H. J. CLULEY remarked that with the old instrument the accuracy for absorptiometric methods was stated by Haywood and Wood to be ±1 per cent. Such an error necessarily limited the use of these methods to the determination of the minor constituents of a material. What was the accuracy claimed for the new model, and could the determination of major constituents of a material be envisaged?

Mr. ISBELL stated that there was no reason why the instrument should theoretically be more accurate than the older model, but as the optical density scale was now uniform and it would therefore be easier to read it, and the instrument could be operated more quickly, it would be found in practice that more accurate readings could be obtained than hitherto.

Mr. J. H. HIGH enquired if "drift" in the Spekker was due to a difference in the *local* illumination of the photo-cells. The light falling on the right-hand cell was the focussed image of the filament, whereas that falling on the left-hand one was not so focussed. On the question of the accuracy of working with the Spekker, it was found some time ago, in determining copper in copper-base alloys, that an accuracy of about 0·2 to 0·3 per cent. of the reading could be readily obtained if great care was taken to control drift.

Mr. ISBELL said that although in theory it might appear that the same area of the photo-cells must be illuminated, it had been found in practice by trying out different optical systems, that it did not affect the drift. With regard to the point on accuracy, many users claim such a figure, but as this depends to some extent on the care of the instrument, the makers would not generally endorse this.

Mr. J. HASLAM asked whether it was correct to say that we were limited to five glass filters of wide waveband. Glass filters of narrow waveband, if at all practicable, would be of great advantage because of the stability of the calibration curves obtained when using them.

Mr. R. L. WOOLLARD remarked that he had found that an accuracy of some measurements could be brought to within about 0·25 to 0·5 per cent. by attention to the following points: (*a*) Frequent re-calibration against standards, as the calibration curve was liable to fluctuate over a period, and greatest accuracy was obtained with the current curve. (*b*) Length of time lamp left on, which should be as short as possible. (*c*) Water-cooling of lamp-house was also useful.

Mr. ISBELL remarked that the information was in agreement with his reply to the second point raised by Mr. High.

Dr. B. BAUMINGER remarked that in their laboratory they had noticed that two cells of the same size, *e.g.*, two 0·5-cm. cells, gave a different drum reading for the same solution under test, and hence the calibration curve changed to about 0·2 and 0·3 per cent. Could Mr. Isbell explain why such a discrepancy should occur and if the cells in general were correctly adjusted.

Mr. ISBELL replied that the two cells in question should be matched. The usual test carried out on the cell was to check first the endplates before manufacture, and then carry out a test on the spacing between the plates after manufacture. The difference between the cells should not be as great as stated, and further investigation seemed to be called for.

Dr. H. A. WILLIAMS suggested that by the use of a simple grating one could make a simple photo-electric spectrophotometer which gave continuous wavelength change, and enabled one to obtain a complete spectrum absorption curve.

Mr. ISBELL remarked that the simple spectrophotometers made with gratings gave a spectral band width as great as, or greater than that transmitted by the Ilford spectrum filters, and there was no particular advantage to be gained by having the continuous variation of wavelength, as the eight pairs of filters covered the spectrum quite sufficiently. The more complicated type of spectrophotometer with a narrow band width came in a different class of instruments altogether, and the price would be prohibitive for the routine work involved.

Mr. A. W. C. MILNER asked Mr. Isbell about the possibility of extending the usefulness of the instrument by using other light sources, such as sodium or cadmium lamps instead of the usual tungsten filament and mercury vapour lamps.

Mr. ISBELL replied that the intensity of these lamps was much lower than that of the high pressure mercury vapour lamp or the tungsten filament lamp, and furthermore, the area of the glowing vapour was so great that the linearity of the drum shutter mechanism would not hold.

Dr. STROSS remarked, with regard to questions raised during the discussion that he had had 4-cm. micro-cells, holding well under 10 ml., made by Tintometer Ltd., and had constructed a cell-holder to fit them. He would be very pleased to send full details to workers interested in these accessories. Furthermore, regarding fluctuations of the calibration graphs, which he had also observed, he thought they had multiple causes, *e.g.*, small variations of the chemical procedure or the reagents or both, ageing of light filters and, particularly, variations of the spectral qualities of the light emitted due to ageing of the lamps and to mains fluctuations. In this respect he wanted to draw attention to a paper by Havemann[3,4] who incorporates a variable resistance of 80 ohms in the lamp circuit of his photometer and adjusts the current so that a standard glass filter, placed in the light path in lieu of the solution, always gives the same standard reading.

Dr. S. Y. THOMPSON said that in his experience one difficulty with the filters in between the cuvette and photo-cell was that most of the glass or gelatin filters tried fluoresce if any of the primary beam (365 mμ.) is reflected on to them, thus setting a limit to the sensitivity to which fluorimeters can be pushed.

Dr. WOKES replied that he had not encountered fluorescence in any of the filters he had tried, at least not sufficient to cause errors.

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Some Experiments with the Spekker Photo-electric Absorptiometer and Fluorimeter

BY F. WOKES AND G. SLAUGHTER

(*Presented at a meeting of the Physical Methods Group on February 22nd*, 1949)

MR. ISBELL[1] has given a very clear picture of the difficulties encountered by the manufacturer when attempting to mass produce an absorptiometer and fluorimeter. We think that Messrs. Hilger & Watts deserve praise for the ingenuity and perseverance with which they are attempting to overcome these difficulties. Our task now is to describe how the various instruments under review stand up to the stress of analytical practice.

Previous work on the Spekker absorptiometer has shown it to give quite satisfactory results in the photo-electric estimation of intensity of colour. Provided that due precautions are taken to avoid turbidity in the solutions examined, the precision obtainable is superior to that experienced with the most careful visual colorimetry. For example, in eighty consecutive assays of nicotinic acid in different foodstuffs, using the original model of the "Spekker", we obtained in our duplicates an average percentage standard deviation (coefficient of variation) for a single assay of 0·88, compared with 1·3 per cent. obtained by Kodicek[2] in fifteen visual estimations of pure nicotinic acid solutions and 3·4 per cent. in sixteen estimations of nicotinamide, using the Zeiss step-photometer but otherwise the same conditions, including the same aromatic amine, as ourselves. Taking into consideration the other sources of error in the assays, we think that in the actual measurement of the colour intensity a difference of 1 per cent. with the "Spekker" would be at least as readily detectable as a difference of 2 to 5 per cent. in visual colorimetry.

Recently we have had the opportunity of testing in our laboratory a prototype of the new model of the Spekker absorptiometer. The larger drum and two-speed control, plus the more rapid lamp-house shutter, should improve the speed and accuracy of the readings. We have found them of much value when examining unstable colours such as occur in nicotinic acid assays. The enlarged holder to take three cuvettes facilitates the comparison of the unknown with two standards, one stronger and the other weaker than the unknown. The increased provision for liquid filters allows greater specificity to be obtained, but it would be an advantage if the filter holders allowed more flexibility in the size of the filters used.

DEVIATIONS FROM LINEARITY IN FLUORIMETRY

Turning next to fluorimetry, this presents special difficulties which can cause serious deviations from linearity—

(*a*) The light energy measured is only 1 or 2 per cent. of the incident light energy,[3] hence minute amounts of stray light produce proportionately large effects.

(*b*) Impurities present may cause "quenching" of the fluorescence.[4] The resulting deviations from linearity can be minimised by using more dilute solutions,[5] but the instrumental blank then forms a larger fraction of the total reading and so increases the deviation from linearity, unless due allowance is made for the blank.

(*c*) The necessity for a continuously operating light source (as compared with intermittent light in absorptiometry) generates heat, which may—
  (i) penetrate to the filters and affect their transmission;
  (ii) reach the liquid in the cuvette and affect its emission of energy.

In the new Spekker fluorimeter, heat from the ultra-violet lamp, which sometimes gave trouble in the old "Spekker," is dissipated by means of improved ventilation, and the eyes of the operator are more effectively protected from the ultra-violet light. The shutter, which

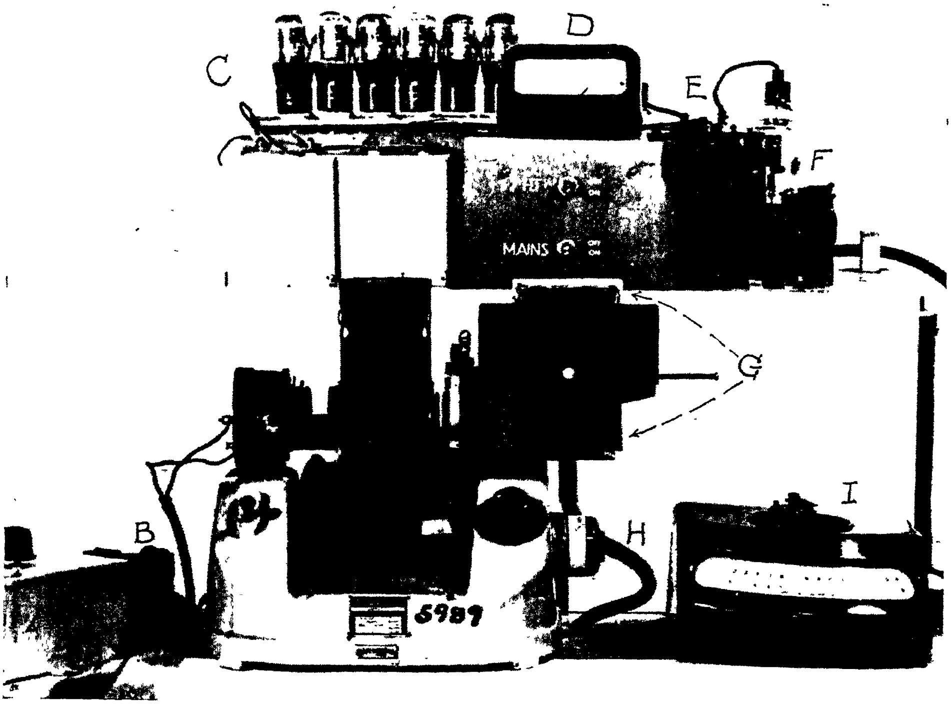
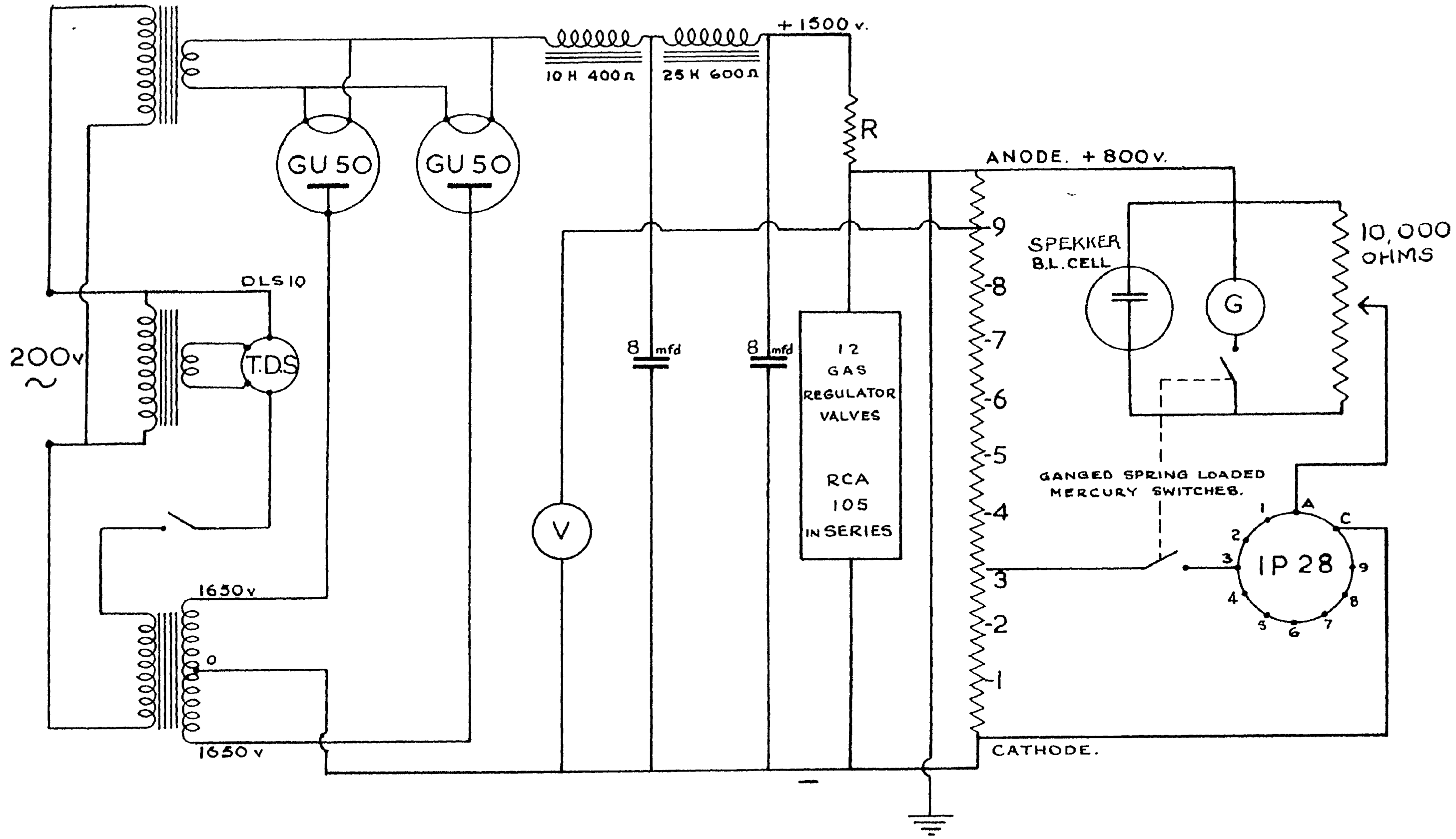


Fig. 1. Illustration of electron multiplier with power pack as applied to Spekker fluorimeter
A. Ganged spring-loaded mercury switches
B. Galvanometer shunt
C. Power pack (12 stabiliser valves in two rows of 6)
D. Dynode voltage indicator
E. High tension transformer
F. High tension filament transformer
G Multiplier housing-adaptor machined to fit original B L. cell cavity
H. Screened multiway polythene cable
I. Cambridge spot galvanometer, used as null point or deflection indicator
C, D, E, and F are normally kept under working bench remote from operator



1P28 MULTIPLIER & POWER SUPPLY CIRCUIT.

Fig. 2. Wiring diagram of electron multiplier with power pack

in the old instrument could become stiff and uncomfortably hot to handle, is considerably improved, but because the galvanometer remains alive it must be guarded against strain when opening the cuvette housing and thus exposing the photo-cells to light. We are glad to note that since our report to the makers on the prototype of the new fluorimeter, steps have been taken to meet this difficulty by adding to the instrument a guard which automatically covers the photo-cells from light when the housing is opened. They have also accepted our suggestion to include more calibrations in the right-hand transmission scale, especially between 70 and 90, thus permitting readings of reciprocal of antilog density to be taken directly, and saving two steps in calculations when allowance is being made for the instrumental blank as described below (p. 625). The linear calibrations in the left-hand density scale are certainly easier to evaluate once one has been accustomed to the "upside-down" scale. Space is available for only one primary filter on the *left*-hand side of the drum, compared with two in the old instrument. On the right-hand side of the drum, space might be contrived for further filters, including a liquid filter for the primary beam (*e.g.*, copper sulphate solution to remove red rays transmitted by Wood's glass). More space also should be available for secondary filters, to meet the demand for narrower band filters, which will increase as their advantages become more widely appreciated.

For analysts interested in riboflavine estimations the most important improvement in the new "Spekker" is probably the marked increase in sensitivity obtained by the use of two large barrier-layer photo-cells. The increase in the photo-receptive area utilised does, however, raise a certain difficulty in that stray light (*e.g.*, reflection of the primary beam from the lid or walls of the cuvette) is afforded more opportunity of reaching the photo-cell. This difficulty can to some extent be overcome by using a mask to cut down the top of the photo-receptive area, and experiments in the use of such masks are now in progress. Even if the primary beam is passed through Wood's glass and highly selective secondary filters are employed, this source of error can exert an appreciable effect in fluorimetric assays of aneurine and riboflavine, especially the latter. Moreover, some barrier layer cells are not satisfactory for measuring very feeble light because of "residual current." The "Spekker" attempts to avoid this by bringing the photo-cells as close as possible to the cuvette, which of course reduces the amount of space available for secondary filters. All these difficulties can be overcome by replacing the barrier-layer photo-cell by a photo-multiplier, obtaining an immense increase in sensitivity which permits the use of a much smaller photo-receptive area at sufficient distance from the cuvette to provide ample space for a variety of secondary filters. Dr. Menzies, of Messrs. Hilger & Watts, has taken much interest in experiments we have been making on the application of photo-tubes to the Spekker fluorimeter, and is considering the possibility of arranging that such photo-tubes can be employed with the new "Spekker."

EXPERIMENTS WITH PROTOTYPE FLUORIMETER

Before describing our experiments with photo-multipliers we should like to present some data obtained with a prototype of the new Spekker fluorimeter, which we were able to test in our laboratories last summer. This prototype was successfully employed in fluorimetric assays of various substances, including aneurine and riboflavine, but we shall refer mainly to the latter vitamin, which presents the greater difficulties. The best method of testing a fluorimeter is to calibrate it with the given fluorescent substance over a sufficiently wide range of concentrations to cover any measurements that may have to be made. Table I summarises data obtained in the calibration of the new "Spekker" with riboflavine solutions matched against a fluorescein standard (referred to as F/1 in the Tables) containing 1 μg. per ml. in phosphate buffer solution of pH 6. The aim is to obtain linearity of response, as shown by a constant ratio between fluorescence intensity and concentration of fluorescent substances. However, in the Spekker instrument, the use of a balanced circuit, whilst bringing certain marked advantages, does mean that the instrumental blank is included in the "density" measured. This involves a departure from linearity becoming more and more marked as more dilute solutions are examined. To overcome this difficulty we took the antilog density for a given concentration and deducted from it the instrumental blank determined on the cuvette filled with the phosphate buffer solution only. In this particular experiment the mean density given by this blank against the fluorescein standard F/1 was 0·869. The antilog of this was 7·396 and the reciprocal of this (0·135) was deducted from each observed fluorescence to obtain the net fluorescence. For the more dilute solutions the zero was set on the riboflavine because its fluorescence was weaker than that of the fluorescein standard, and the reciprocal of the antilog density was, therefore, employed to calculate the net fluorescence. This net fluorescence when divided by the concentrations gave a fluorescence/concentration ratio which (apart from the concentration quenching with the three strongest solutions) remained remarkably constant between 2·74 and 2·75 over the range from ¼ to 1/32 μg. per ml. The linearity thus obtained compared favourably with that found by Umberger and LaMer,[6] who used fluorescein solutions, the fluorescence of which was similar to that of our weakest riboflavine solutions, in a fluorimeter that they claimed to be more sensitive than any so far described in the literature.

TABLE I

CALIBRATION OF NEW SPEKKER FLUORIMETER WITH RIBOFLAVINE SOLUTIONS AGAINST FLUORESCEIN STANDARD F/1 (1 μG./ML.)

Riboflavine μg./ml.	Mean "density" against F/1	Antilog density (AD)	$\frac{1}{AD}$ = RAD	RAD − blank = net fluorescence	$\frac{\text{Fluorescence}}{\text{Concentration}}$
F/1 at zero					
4	0·833	6·806		6·67	1·67
2	0·634	4·305		4·17	2·09
1	0·417	2·612		2·48	2·48
Riboflavine at zero					
¼	0·086	1·219	0·821	0·686	2·74
⅛	0·320	2·089	0·479	0·344	2·75
1/16	0·514	3·262	0·307	0·172	2·75
1/32	0·656	4·529	0·221	0·086	2·75

VARIATION IN INSTRUMENTAL BLANKS

When the Spekker fluorimeter is calibrated in the manner we have just described, the degree of linearity obtained depends largely on the accuracy with which the instrumental blank can be determined. Table II shows how this blank can vary widely with different cuvettes, and with the same cuvette used under different conditions. In a series of five cuvettes supplied with ground glass lids the instrumental blank in riboflavine estimations, whilst varying somewhat between different estimations, was on the whole much higher than in a series of three cuvettes with clear glass lids. This was probably due to the reflection from the ground glass lids of red rays which were transmitted by the secondary filters used in riboflavine estimations, because when the primary Wood's glass filter was supplemented by a liquid copper sulphate filter cutting out the red rays, the blanks were much reduced. When cuvettes with clear glass lids were used, the inclusion of the H503 filter to exclude heat rays produced on the whole rather smaller reductions in the blanks. Still smaller blanks are obtained by using black glass lids.

TABLE II

INSTRUMENTAL BLANKS IN RIBOFLAVINE ESTIMATIONS WITH NEW SPEKKER FLUORIMETER

	Blank as % of F/1, using cuvettes with lids of	
Primary filter	Ground glass	Clear glass
Wood's	20, 18, 19, 18	9, 9, 15
	16, 18, 22, 23	16, 12, 14
	20, 18, 19	
	22,* 26,† 18**	11,†† 19§
Wood's + copper sulphate	13,* 15†	
Wood's + H503	13**	10,†† 8§
Wood's + large H503		8,†† 6§

* These results obtained with the same cuvette.
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APPLICATION OF PHOTO-MULTIPLIERS TO SPEKKER FLUORIMETER

In some estimations, *e.g.*, that of riboflavine, it is desirable to employ rather low concentrations of fluorescent substances. These give with barrier-layer photo-cells insufficient outputs to provide satisfactory galvanometer responses. If the latter are amplified optically, by increasing the distance between the light and the mirror, serious difficulties can arise because of vibrational effects. Vacuum-type photo-cells and amplifiers with high grid resistance tend to be rather temperamental under normal working conditions. These difficulties are obviated by the use of photo-multipliers, with their more easily stabilised high voltage supply. The enormous increase in sensitivity obtained with these photo-multipliers opens out such possibilities in fluorimetry that their widespread employment may eventually be expected. During the past two years we have been experimenting with a variety of photo-multipliers, both American and British types. Fig. 1 shows our set-up as applied to an old Spekker fluorimeter originally purchased in 1941. In this set-up, which has been in regular use in our laboratory for nearly a year, the photo-multiplier is mounted in a light-tight box attached to the back of the cuvette housing. The fluorescent light enters this box through an aperture about 1 cm. in diameter, which is in the position formerly occupied by the barrier-layer photo-cell. On striking the photo-receptive area on the cathode it produces a current which is amplified about 45,000-fold, using the present dynode voltage of 80 volts per stage. Provision has been made for operating at 125 volts per stage, which will increase the current amplification to about 800,000. The enormous increase in sensitivity thus obtained permits the use of a stable spot galvanometer in place of the mirror galvanometer previously employed, and thus obviates any need for anti-vibration devices. The dynode voltage per stage is measured by a voltmeter permanently in circuit for check purposes and has been found to remain remarkably constant. Fig. 2 shows the wiring diagram.

A difficulty in multiplier use is effective regulation of the high voltages required. We have employed a series of twelve R.C.A. 105 gas diodes with a potential of 1260 volts across them. Constancy to a quarter of a volt per stage is maintained for main input changes of 180 to 230 volts A.C., a control of potential sufficiently close to provide satisfactory results.

These gas diodes used as stabilisers provided a very simple solution to the problem of dynode voltage regulation and, although relatively cumbersome compared with electronic methods, have proved remarkably stable during over a year of constant usage.

To facilitate the measurement of very faint fluorescence we adopted the expedient of "backing off" the blank deflection by means of the left-hand photo-cell of the "Spekker," applying its output in reverse and returning the galvanometer to zero by optical light control with the iris diaphragm. This enabled us to measure the fluorescence of very dilute quinine and fluorescein solutions, down to a thousandth of a microgram per millilitre, which is considerably lower than has been previously reported.

Table III gives some calibration data obtained with this old "Spekker" plus photo-multiplier, and shows that the degree of linearity, as measured by the fluorescence/concentration ratio, compared favourably with that given by the new "Spekker."

TABLE III

CALIBRATION OF OLD SPEKKER FLUORIMETER PLUS PHOTO-MULTIPLIER AGAINST FLUORESCEIN STANDARD F/1 (1 µG./ML.)

Riboflavine µg./ml.	Mean "density" against F/1	Antilog density (AD)	$\frac{1}{AD}$ = RAD	RAD − blank = net fluorescence	$\frac{\text{Fluorescence}}{\text{Concentration}}$
F/1 at zero					
1	0·258	1·809		1·77	1·77
½	0·004	1·009		0·968	1·94
Riboflavine at zero					
¼	0·250	1·778	0·562	0·521	2·08
⅛	0·520	3·311	0·302	0·261	2·09
1/16	0·760	5·754	0·174	0·133	2·13
1/32	0·980	9·55	0·105	0·064	2·05

COMPARISON OF FLUORIMETERS

In the comparison of fluorimeters for analytical purposes two main factors must be considered—

(*a*) sensitivity;
(*b*) instrumental blank.

Table IV gives data on these factors for the old "Spekker," the new "Spekker" and the old "Spekker" plus R.C.A. I.P.28 photo-multiplier, when used in aneurine and riboflavine estimations. It will be seen that the new "Spekker" has 5 to 7 times the sensitivity of the old "Spekker," and that the addition of the photo-multiplier to the old one increases its sensitivity 63-fold in riboflavine estimations, and 75-fold in aneurine estimations. The instrumental blanks are all only 1 to 3 per cent. of the quinine and fluorescein standards, except in riboflavine solutions with the new "Spekker," when they reach 10 per cent. or more. This is due largely to reflection from the cuvette lids of red light in the incident beam, because when the clear glass lids are replaced by black glass lids, absorbing this light, the instrumental blank in riboflavine estimations is considerably reduced.

TABLE IV

COMPARISON OF FLUORIMETERS

	Relative sensitivity for estimating		Instrumental blanks as % of	
	Aneurine	Riboflavine	Quinine standard Q/1	Fluorescein standard F/1
Old "Spekker"	1	1	2	3
New "	5	7	1–2	10
Old " plus I.P.8 photo-multiplier	75	63	2–3	1½–2

NOTE—

All estimations made using Tinsley galvanometer and normal set-up of filters, etc. Photo-multiplier used at only 78 volts per stage.

Later results indicate that the R.C.A. I.P.21 photo-multiplier has definite advantages over the I.P.28 photo-multiplier in riboflavine estimations, as we hope to show in a further publication.

The instrumental blank is due mainly to unwanted reflection from cuvette lid and walls of improperly filtered light (primary or fluorescent or both). Improvements will therefore come from—

(*a*) reducing the photo-cell aperture to minimise the amount of unwanted reflection that reaches the photo-receptive area; and

(*b*) better control of filtering properties both in the primary beam and in the fluorescent light.

Different instruments may vary in the relative importance of (*a*) to (*b*), and the instrument will give different results when the exciting light wavelength or the fluorescent light band is changed.

The greatest improvements in regard to blanks will probably come from the use of—

(*a*) a copper sulphate filter in the primary beam to eliminate red rays when using red-sensitive detectors such as barrier-layer cells;

(*b*) narrower cut secondary filters transmitting practically none of the primary light used;

(*c*) photo-multiplier tubes, giving extra sensitivity which will allow of (*b*) and of small apertures.

APPLICATION OF FLUORESCENCE SPECTRA

We have recently been employing fluorescence spectra to explore the disturbing factors responsible for these instrumental blanks. Light from the Spekker ultra-violet lamp, after being filtered through Wood's glass, was passed into the cuvette containing the liquid under examination and the radiation from the cuvette was taken at right-angles into a Hilger wavelength spectrometer with camera attachment. The fluorescence spectra thus obtained indicated that the light from the instrumental blank is most intense in the spectral region corresponding to the fluorescein fluorescence used in riboflavine assays, and much weaker in the spectral region corresponding to the quinine fluorescence used in aneurine assays. Thus an explanation is provided for the higher blanks observed with the "Spekker" in riboflavine assays. These high blanks can be considerably reduced by altering the cuvette lids, and especially by cutting down the size of the photo-sensitive surface employed to detect the fluorescence, in which the use of photo-multipliers will be very important.

The spectrometer has also been used to investigate the nature of the incident beam obtained when Wood's glass is used as primary filter. It was found that an appreciable amount of visible light was passing through the Wood's filter. Since this provided the possibility of obtaining readings too high in fluorimetric assays, especially of riboflavine, experiments have been carried out on the replacement of the Wood's filter by Wratten 39 and 47 filters, in the presence of a H503 heat-resiting filter. These enabled us to obtain fluorimetric results in much closer agreement with microbiological assays on the same samples, containing a good deal of interfering substances. Full details of these experiments will be published later.

Spectroscopic methods can be used to check the efficiency of various analytical procedures and should lead to further advances in the science of fluorimetry.

We are indebted to Mr. E. J. Bowen, F.R.S., for advice, and to Miss Chloe Klatzkin for numerous data on nicotinic acid and riboflavine estimations.

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Polarographic Determination of Ethylenediamine-Tetra-acetic Acid

BY W. FURNESS, P. CRAWSHAW AND W. CULE DAVIES

THE sodium salt of ethylenediamine-tetra-acetic acid*:

$$(HOOC.CH_2)_2N.CH_2.CH_2.N(CH_2COOH)_2$$

known as Trilon B (I.G. Farbenindustrie A.-G.), forms with certain metals soluble complex salts in which the metal exists in a non-ionised form. The compound can be considered as the organic counterpart of inorganic sequestering agents, such as Calgon, and is used in many industries, for example, for delaying or preventing the precipitation of calcium. The commercial product consists of the sodium salt of the diamine-acid mixed with sodium sulphate or sodium carbonate. A method for the evaluation of sequestrants has been described by Hart[1] and modified by Zussmann,[2] who titrated the agent with a soluble calcium salt in presence of sodium oxalate as indicator, the end-point being given when a standard turbidity was reached. The stability of the copper and calcium complexes of the diamine-acid were measured polarographically by Pfeiffer and Offermann,[3] but no details or results were given and the investigation was not extended to analytical purposes. In a paper published since the completion of our investigation, Souchay and Faucherre[4] have shown how Trilon B may be employed for the polarographic determination of cobaltic and ferric ions.

In the present paper the polarographic determination of Trilon B has been investigated by making use of the ability of ethylenediamine-tetra-acetic acid to form complexes with ferric or cupric ions. For a quantitative polarographic analysis of the diamine-acid, an excess of ferric or cupric salt is added and the problem then is to determine the concentration of the metallic complex of the diamine-acid in presence of the excess metallic ion. Several procedures have been tried and it was found that the copper complex is the more useful for quantitative analysis, but the ferric complex has value in qualitative work.

Standard solutions—The purity of a re-crystallised specimen of ethylenediamine-tetra-acetic acid (m.p. 241·5° C.) was checked by potentiometric titration with standard sodium hydroxide, using a hydrogen electrode and a saturated calomel electrode. 2·636 g. of this specimen of the acid and 135 ml. of 0·2 *N* sodium hydroxide diluted to 500 ml. gave a solution of the trisodium salt of concentration 0·0180 *M*.

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* Frequently referred to below as "the diamine-acid," for brevity.

0·0180 *N* Ferric ammonium sulphate in 0·1 *N* sulphuric acid and 0·0180 *M* copper sulphate in water were also prepared.

FERRIC COMPLEX OF ETHYLENEDIAMINE-TETRA-ACETIC ACID

(*a*) *Direct separation of the waves due to the complex and to ferric ion*—In a supporting electrolyte of potassium nitrate, ferric ion gives a cathodic current ($Fe^{\cdot\cdot\cdot} \rightarrow Fe^{\cdot\cdot}$) at the dropping mercury electrode, the limiting current at +0·2 volt *versus* the Saturated Calomel Electrode (S.C.E.) being proportional to its concentration. With an excess of ethylenediamine-tetra-acetic acid present, the well defined wave due to the ferric complex of the diamine-acid begins at +0·1 volt *versus* S.C.E. and, so long as the molarity of the diamine-acid is equal to or greater than the normality of the ferric iron, the limiting cathodic current is proportional to the concentration of ferric iron (since the diamine-acid itself gives no polarographic wave). If the normality of ferric iron exceeds the molarity of the diamine-acid, as in the example given in Fig. 1, the cathodic current at +0·2 volt *versus* S.C.E. is proportional to the excess of iron and the cathodic step beginning at +0·1 volt *versus* S.C.E. is proportional to the concentration of the diamine-acid. Proceeding thus, it was shown that one ferric ion is associated with one molecule of the diamine-acid in the complex. For a given concentration of ferric iron the total cathodic current at —0·2 volt is almost independent of the concentration of the diamine-acid, confirming that the cathodic wave beginning at +0·1 volt *versus* S.C.E. is due to the reduction of the complexed ferric ion to the bivalent state.

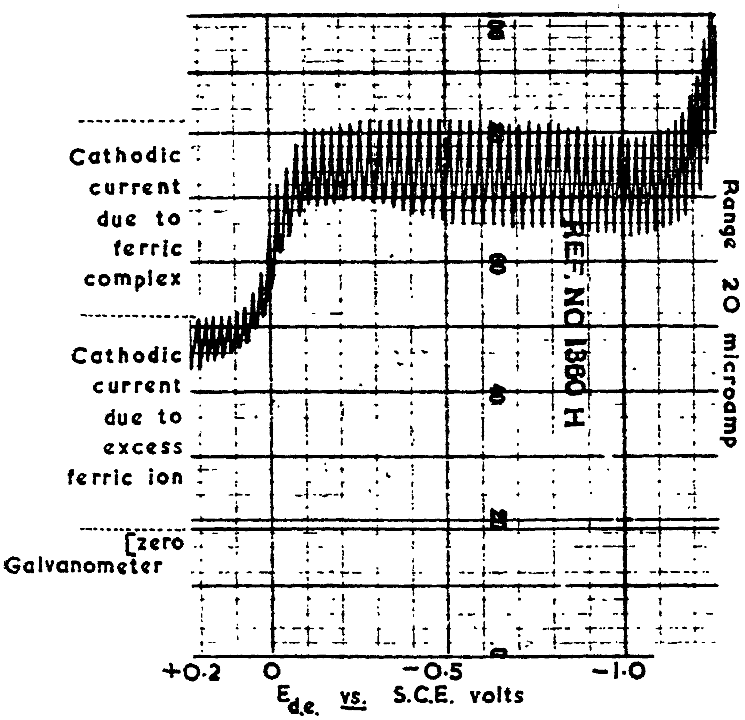

Fig. 1. Polarogram of the solution—
5 ml. 0·0180 *M* $Na_3C_{10}H_{13}O_8N_2$,
10 ml. 0·020 *N* ferric ammonium sulphate,
10 ml. *N* potassium nitrate,
2·5 ml. 0·1 per cent. gelatin,
after dilution to 50 ml.
Temperature 25·0° C. $m^{2/3}t^{1/6} = 1{\cdot}92$ mg.$^{2/3}$sec.$^{-1/2}$

In the ideal case illustrated in Fig. 1, the diffusion current of the ferric complex of ethylenediamine-tetra-acetic acid can be measured precisely as the difference between the total diffusion current at —0·2 volt and that at +0·2 volt due to the excess of ferric ion.

The latter, however, can be measured accurately only in the absence of anions, such as chloride, which themselves give anodic waves at +0·2 volt.

(*b*) *Precipitation of the excess of ferric ion*—Ammonia and an aqueous suspension of zinc oxide both cause some decomposition of the ferric complex of the diamine-acid, but hexamethylenetetramine is a promising reagent, for it precipitates the excess of ferric ion without causing decomposition of the ferric complex of the diamine-acid. Slight occlusion of the ferric complex of the diamine-acid on the precipitated ferric hydroxide was, however, observed. An example of the procedure is as follows.

Treat 5 ml. of 0·0180 *M* sodium salt of the diamine-acid with an excess (10 ml.) of 0·0180 *N* ferric ammonium sulphate, add 10 ml. of *N* ammonium nitrate, 2·5 ml. of 0·1 per cent. gelatin solution and 10 ml. of *M* hexamethylenetetramine and dilute to 50 ml. Filter through a dry paper to remove the precipitated ferric hydroxide and measure the cathodic current due to the ferric complex of the diamine-acid in the filtrate.

(*c*) *Removal of excess of ferric ion by further complex formation*—On theoretical grounds it appeared unlikely that a suitable complexing agent could be found, and experiments that were performed confirmed this.

(*d*) *Reduction of the excess of ferric ion*—The use of hydrazine sulphate or hydroxylamine hydrochloride in attempts to reduce the excess of ferric ion whilst leaving the ferric complex of the diamine-acid unaffected was investigated. It was found that the ferric complex of the diamine-acid was also partly reduced.

CUPRIC COMPLEX OF ETHYLENEDIAMINE-TETRA-ACETIC ACID

The addition of 5 ml. of 0·0180 *M* cupric sulphate to 5 ml. of 0·0180 *M* solution of the sodium salt of the diamine-acid gives an intensely blue solution due to the cupric complex which, in a supporting electrolyte of 0·2 *N* ammonium or potassium nitrate containing 0·005 per cent. of gelatin, gives a well defined cathodic wave with half-wave potential —0·2 volt *versus* S.C.E. If the diamine-acid is present in excess, only the single wave of the cupric complex is recorded, its height being proportional to the quantity of cupric sulphate taken. On the other hand, when excess of cupric ion is present, the reduction wave of the cupric complex cannot effectively be separated from that due to the reduction of the simple ion $Cu^{\cdot\cdot} \rightarrow Cu$. It can be shown that each molecule of ethylenediamine-tetra-acetic acid is co-ordinated to one atom of copper.

The limiting value of the cathodic current of the cupric complex of the diamine-acid is approximately equal to the total diffusion current ($Cu^{\cdot\cdot} \rightarrow Cu$) of an equivalent solution of cupric sulphate. The polarographic wave of the cupric complex of ethylenediamine-tetra-acetic acid must, therefore, be due to the reduction of the co-ordinated copper to zero valency state.

Two methods of separating the polarographic waves due to the cupric complex of the diamine-acid and to the excess of cupric copper have been investigated.

(*a*) *Direct polarographic determination of the cupric complex in presence of excess of copper*—In ammoniacal solution the reduction wave of the cupric complex of ethylenediamine-tetra-acetic acid is shifted to more negative potentials and is preceded by the waves due to the two-stage reduction of the cuprammonium ion if excess of cupric sulphate has been taken. Fig. 2 shows, for example, a polarogram of a solution of the copper complex of the diamine-acid with an excess of cupric ion in an ammoniacal ammonium nitrate supporting electrolyte. The wave due to the first stage reduction of the cuprammonium ion can be measured accurately. The height of the second wave due to reduction in the second stage to zero valency must be equal to that of the first wave. This second wave, however, coalesces with a third wave, due to reduction of the cupric complex of the diamine-acid. Although it is not possible to measure the third wave precisely, the diffusion current due to the complex can be computed approximately by deducting from the total current at —1·0 volt *versus* S.C.E. a value equal to twice the diffusion current at —0·25 volt *versus* S.C.E.

(*b*) *Precipitation of excess of copper by means of magnesium oxide*—A solution containing 5 ml. of a 0·0180 *M* solution of the sodium salt of the diamine-acid, 5 ml. of 0·0180 *M* cupric sulphate, 10 ml. of *N* potassium nitrate and 2·5 ml. of 0·1 per cent. gelatin solution was treated in the cold with 10 ml. of 5 per cent. magnesium oxide suspension, then diluted to 50 ml. and filtered through a dry paper. The filtrate gave a polarogram reproduced in Fig. 3, in which the limiting cathodic current is 10·7 microamp. Since this value is identical with that obtained for another solution prepared as above but without the treatment with magnesium oxide, it is inferred that in cold solution magnesium oxide causes no decomposition of the copper complex of the diamine-acid.

Taking 10 ml. of 0·0180 *M* cupric sulphate, that is, 100 per cent. excess, in the above solution and carrying out the treatment with magnesium oxide, the filtrate gave a diffusion current of 10·6 microamp. Thus, no significant quantity of the copper complex of the diamine-acid is occluded in the magnesium oxide - copper hydroxide precipitate. The half-wave potential of the copper complex in this supporting electrolyte is at about —0·44 volt *versus* S.C.E., and thus there can be little interference with the wave form due to the presence of chloride ion.

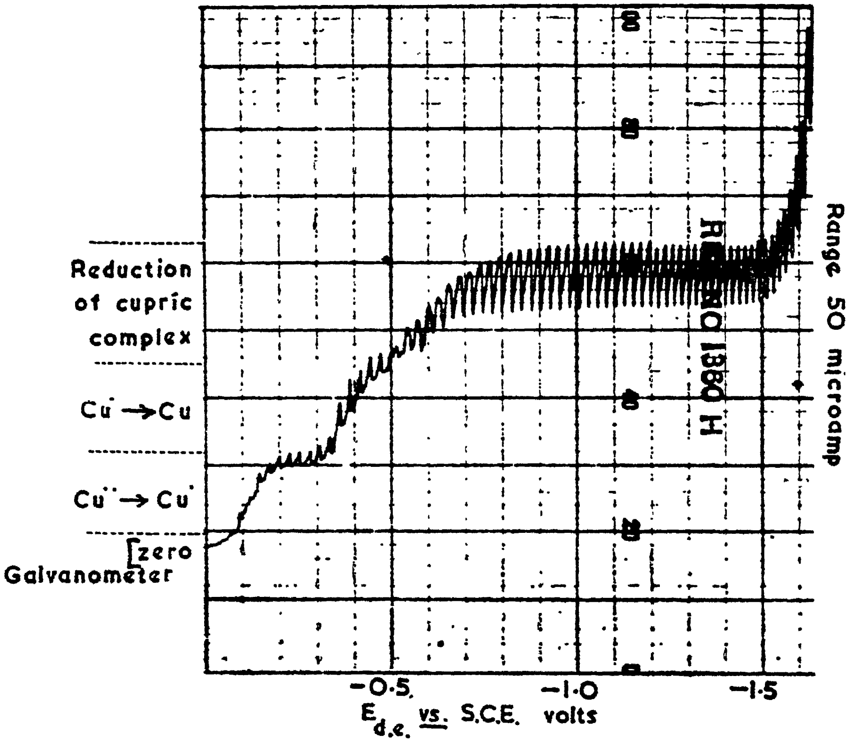

Fig. 2. Polarogram of the solution—

5 ml. 0·0180 *M* $Na_3C_{10}H_{13}O_8N_2$,
10 ml. 0·0180 *M* cupric sulphate,
10 ml. *N* ammonium nitrate,
10 ml. *N* ammonium hydroxide,
5 ml. 0·1 per cent. gelatin solution,
after dilution to 50 ml.
Temperature 25·0° C. $m^{2/3}t^{1/6} = 1·92$ mg.$^{2/3}$sec.$^{-1/2}$

Standardisation—Prepare a series of standard solutions of the cupric complex of ethylene-diamine-tetra-acetic acid using a 10 per cent. excess of cupric sulphate in each case. Add potassium nitrate and gelatin so as finally to give a supporting electrolyte of 0·2 *N* potassium nitrate containing 0·005 per cent. of gelatin, treat with excess of magnesium oxide suspension, dilute to standard volume and filter. Measure the diffusion currents at —0·7 volt *versus* S.C.E. (*for maximum drop size* at 25·0° C. and corrected for residual current, using a Tinsley fast pen-recording polarograph *without damping*). For solutions of the cupric complex up to a concentration of at least 5 millimolar the limiting diffusion current is directly proportional to the concentration. When the rate of flow of mercury from the capillary is 1·957 mg. per second and the drop time at —0·7 volt *versus* S.C.E. is 3·42 seconds ($m^{2/3}t^{1/6} = 1·92$ mg.$^{2/3}$sec.$^{-1/2}$), the mean value of i_d/C is 5·96 microamp. millimole.$^{-1}$ litre. The probable error of this mean was 0·04 microamp. millimole.$^{-1}$ litre.

Determination of the half-wave potential of the cupric complex of ethylenediamine-tetra-acetic acid—The pH of the 0·2 *N* potassium nitrate, 0·005 per cent. of gelatin supporting electrolyte after treatment with magnesium oxide lies between 6·5 and 7·0. At this pH manual polarograms of the cupric complex of ethylenediamine-tetra-acetic acid have been plotted for two solutions of differing concentration. The potential of the dropping mercury electrode at each point on the wave was measured with a vernier potentiometer by reference to the saturated calomel electrode at 25° C. for *maximum drop size*, using a method (shortly to be published) which eliminates the need for corrections for the *i*R drop between the working electrodes of the polarographic cell. The corresponding values of the diffusion current at maximum drop size were recorded by means of a Tinsley fast pen-recorder. The values obtained for the half-wave potentials ($E_{1/2}$) of 0·00451 *M* and 0·00135 *M* solutions of the cupric complex were, respectively, —0·445 volt and —0·442 volt *versus* S.C.E.

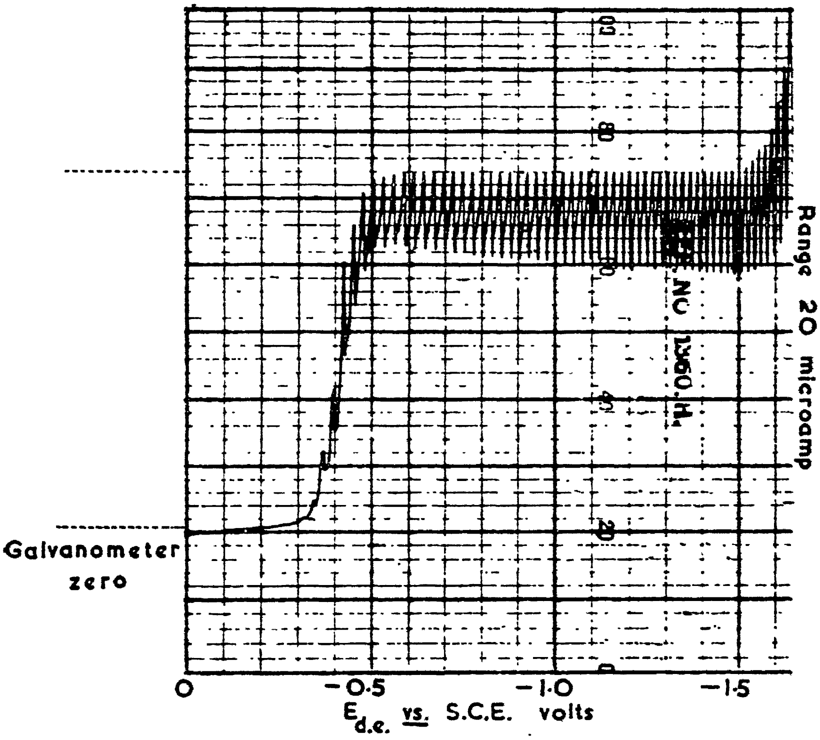

Fig. 3. Polarogram of 0·00180 *M* cupric complex of ethylene-diamine-tetra-acetic acid in 0·2 *N* potassium nitrate, 0·005 per cent. gelatin after treatment with magnesium oxide

Temperature 25·0° C. $m^{2/3}t^{1/6} = 1·92$ mg.$^{2/3}$sec.$^{-1/2}$

The experimental data were also used to make a plot of log $i/i_d - i$ against $E_{d.e.}$, where i_d is the limiting value of the diffusion current, and i the diffusion current when the potential of the dropping electrode is $E_{d.e.}$ As shown in Fig. 4 this log plot is linear, and shows that the equation of the polarographic wave is given by

$$E_{d.e.} = -0·444 - \frac{RT}{0·81F} \log_e \frac{i}{i_d - i}$$

DETERMINATION OF ETHYLENEDIAMINE-TETRA-ACETIC ACID IN MIXTURES OF ITS SODIUM SALTS WITH SODIUM CARBONATE AND SULPHATE

A quantitative method is based on the conversion of the diamine-acid into its cupric complex, which is determined polarographically, after the constant i_d/C for the particular capillary being used has been first determined.

When the ethylenediamine-tetra-acetic acid content is known approximately, the amount of copper required to form the complex can be calculated, and a quantity of cupric sulphate 10 to 20 per cent. in excess is added. If the content of the diamine-acid is unknown, and if it should later be found that the copper added is a tenfold or more excess, slight occlusion of the complex on the precipitated copper hydroxide occurs. The analysis is then repeated, using less cupric sulphate. So long as the copper taken is not more than a fivefold excess, the relative error due to occlusion by the precipitate is less than 2 per cent.

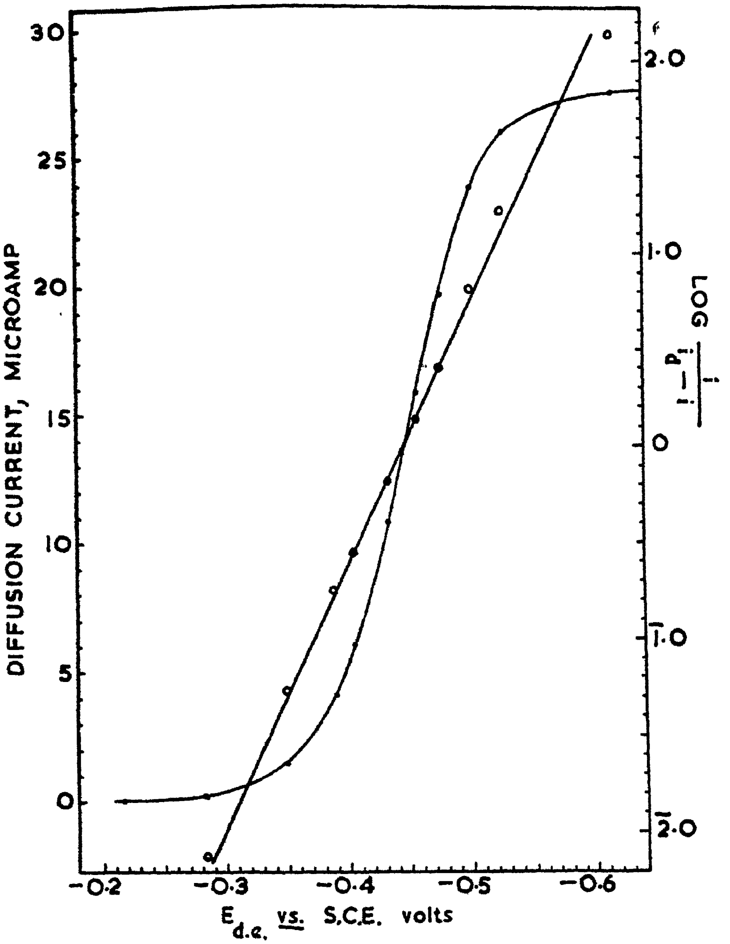

Fig. 4. Manual polarogram of 0·00451 *M* solution of the cupric complex of ethylenediamine-tetra-acetic acid in 0·2 *N* potassium nitrate, 0·005 per cent. gelatin, after treatment with magnesium oxide, and log plot of the wave

Temperature 25·0° C. $m^{2/3}t^{1/6} = 1{\cdot}92$ mg.$^{2/3}$sec.$^{-1/2}$

Transfer 0·2 g. of the sample to a 100-ml. graduated flask, and dissolve in 20 ml. of cold water. Using methyl red as indicator, neutralise the solution with dilute sodium hydroxide solution or dilute nitric acid as may be required. Add 20 ml. of 0·02 *M* cupric sulphate (which is sufficient to react with an ethylenediamine-tetra-acetic acid content of 58 per cent.), mix thoroughly, then add 20 ml. of *N* potassium nitrate, and precipitate the excess of copper by addition of 10 ml. freshly prepared 5 per cent. aqueous suspension of magnesium oxide. Add 5 ml. of 0·1 per cent. gelatin solution, dilute to 100 ml., mix well, and immediately filter through a dry Whatman No. 40 paper. Obtain a polarogram of the filtrate between 0 and —1·0 volt *versus* S.C.E., and measure the diffusion current at —0·7 volt *versus* S.C.E. From the expression $i_d/C = K$ calculate C, and thence the percentage of ethylenediamine-tetra-acetic acid in the sample.

In this way a commercial sample of Trilon B was found to have a content of 22·4 per cent. of ethylenediamine-tetra-acetic acid. An analysis by a photometric method, based upon a comparison of the optical densities of the golden-yellow solutions of the ferric complex, also gave the result 22·4 per cent. for the ethylenediamine-tetra-acetic acid content of the sample. By the Kjeldahl method, however, the nitrogen content of the sample was found to be 2·30 per cent., which corresponds to 24·0 per cent. of ethylenediamine-tetra-acetic acid. The discrepancy is considered too large to be wholly attributable to experimental error, and when pure ethylenediamine-tetra-acetic acid was added to this commercial sample the result of polarographic analysis was in satisfactory agreement with the standard addition. It must be borne in mind that the sample might well have contained traces of nitrogen compounds other than ethylenediamine-tetra-acetic acid. By the application of the procedure reported by Zussmann[2] we failed to obtain results of any precision.

SUMMARY

The polarographic characteristics of the ferric and cupric complexes of ethylenediamine-tetra-acetic acid have been investigated in order to provide a polarographic method for the evaluation of commercial samples of the sodium salt of ethylenediamine-tetra-acetic acid (Trilon B). The properties of the cupric complex were found to be particularly suited for this purpose, and a quantitative method of analysis has been developed in which the complex, formed in presence of an excess of cupric ion, is determined polarographically after precipitating the excess of copper with magnesium oxide.

In the analysis of a commercial sample of ethylenediamine-tetra-acetic acid the polarographic result has been compared with those obtained by an independent photometric procedure and by the Kjeldahl method.

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CENTRAL RESEARCH DEPARTMENT
BROTHERTON AND CO. LTD., LEEDS

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Amperometric Titration of Copper and Ferric Iron with Cupferron*

BY I. M. KOLTHOFF AND A. LIBERTI†

CUPFERRON, the ammonium salt of nitrosophenylhydroxylamine, has been introduced by Baudisch[1] as a reagent for the quantitative determination of copper and ferric iron. Later it appeared that a number of metals react with cupferron, which is a useful but unspecific reagent. Its use has been confined mainly to gravimetric analyses and separations. de Brouckère and Belcke[3] developed a nephelometric method for the determination of traces of aluminium, and recently Corwin and Meyer[4] found cupferron a satisfactory reagent in the conductimetric titration of copper.

The present paper reports the results of a study of the amperometric titration of copper and ferric iron when present singly and in a mixture of both, with the dropping mercury electrode as the indicator electrode.

The polarography of cupferron at various pH values has been described in a recent paper.[6] Dependent upon the pH, its diffusion current is found at a relatively negative potential. For this reason we prefer in general to carry out the titration of copper and iron at a potential at which these metals yield a diffusion current but cupferron is not reduced.

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* From a Master's thesis submitted by A. Liberti to the Graduate School of the University of Minnesota, 1947.

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STABILITY OF CUPFERRON SOLUTIONS—

Biltz and Hödtke[2] claim that an aqueous solution of the reagent can be stored unaltered for several weeks. Rodejo[11] reports increasingly low results in the precipitation of vanadium with increasing age of cupferron solutions. Pinkus and Martin[9] recommend the use of freshly prepared solutions; on standing, such solutions develop a yellowish colour, and an aromatic odour like that of nitrobenzene becomes noticeable. We prepared two sets of solutions from purified cupferron; one was 0·2 *M* and the other 0·02 *M*. At both molarities the solutions were stored in the following media: in conductivity water (pH 6·3); in buffer solution containing 0·1 *M* pyridine and 0·05 *M* hydrochloric acid (pH 4·4); in buffer solution containing 0·1 *M* ammonia and 0·1 *M* ammonium chloride (pH 9·5); and in 0·1 *M* ammonia. The solutions were stored over a period of two weeks and the decomposition was followed by measuring the diffusion current of the diluted solutions in a biphthalate buffer having pH 5·2. Although the solutions in water and in ammoniacal medium at both molarities of the reagent became yellow and developed an aromatic odour, no indication of decomposition was obtained from polarographic measurements. The solutions kept at pH 4·4 decomposed rapidly; after 8 hours the concentration had decreased to one half its original value and after two days the decomposition was complete. The rate of decomposition was found to increase with decreasing pH; in 0·01 *N* hydrochloric acid the decomposition was marked after half an hour. Addition of 50 mg. of *p*-acetophenetidine per 150 ml. of solution, recommended by Germuth[5] as a stabiliser, did not have a noticeable effect in any of the stock solutions investigated. When kept in the dark and in an ice-box the solutions in water and in ammoniacal medium remained colourless and developed no odour in a period of two weeks. For analytical purposes standard solutions of cupferron should be prepared in conductivity water and kept in dark bottles. They can be used without change of titre over a period of two weeks.

AMPEROMETRIC TITRATIONS

In the titration of copper a 0·02 *M* and in the titration of ferric iron a 0·03 *M* solution of the reagent was prepared from a commercial product of cupferron that had been recrystallised from alcohol. The crystals could be kept unchanged when stored in the dark over ammonium carbonate. The stock solutions can be standardised against standard copper or ferric iron solutions by the procedures given below. When properly prepared the stock solutions of the reagent were found to have the calculated normality.

The amperometric titrations with the dropping mercury electrode as indicator electrode were carried out by the same general technique as described in previous publications.[7] Oxygen was removed by means of nitrogen. After each addition of reagent nitrogen was bubbled through vigorously; this promoted the coagulation of the very finely divided precipitate which was formed. The current measurements were more or less irregular when most of the precipitate stayed in colloidal suspension. A saturated calomel electrode was used as an outside reference electrode and the potentials refer to this electrode. In general, the titrations were carried out at potentials at which copper or ferric iron yields a diffusion current, but at which cupferron is not reduced. Thus, a type of titration diagram was obtained as shown in Fig. 1 *a*. If the titration was carried out at a potential at which both the metal ion and the reagent yield a diffusion current a titration diagram of the type of Fig. 1 *b* was obtained. In general, it is disadvantageous to carry out the titration at a potential at which cupferron is reduced. In the first place, several metals that are not reduced at potentials at which copper and iron yield diffusion currents are reduced at potentials at which cupferron gives a diffusion current. Moreover, the current due to dissolved metal cupferronate at and near the end-point becomes much greater when the titration is carried out at a potential where cupferron yields a diffusion current than at a potential where only copper or iron give a diffusion current. It should be remembered that one ferric ion, which gives a one-electron reduction, combines with 3 cupferron molecules, corresponding to a reduction involving 3 × 6 electrons. Incidentally, it may be mentioned that the polarographic method should be suitable for the determination of the solubility of metal cupferronates under various conditions at a potential at which cupferron yields a diffusion current.

TITRATION OF CUPRIC COPPER

Procedure—Place a measured volume of the copper solution in the titration cell and add a phthalate, citrate, acetate or tartrate buffer with a pH between 5 and 6. The final concentration of the buffer in the mixture is 0·05 *M* or less. Add gelatin to give a concentration of 0·01 per cent., remove oxygen by bubbling nitrogen through the solution and apply a potential of —0·5 volt. Titrate with 0·02 *M* cupferron solution and pass a vigorous stream of nitrogen through after each addition of reagent to promote coagulation of the bulky precipitate. The end-point, which is found graphically in the usual way, corresponds to a reaction between one mole of cupric copper and two moles of cupferron.

Discussion—An example of the titration diagram obtained with the use of a citrate buffer is given in Fig. 1 *a*. When the titration was carried out at a potential of —1·45 volt, at which both copper and cupferron yield diffusion currents, a titration diagram as in Fig. 1 *b* was obtained.

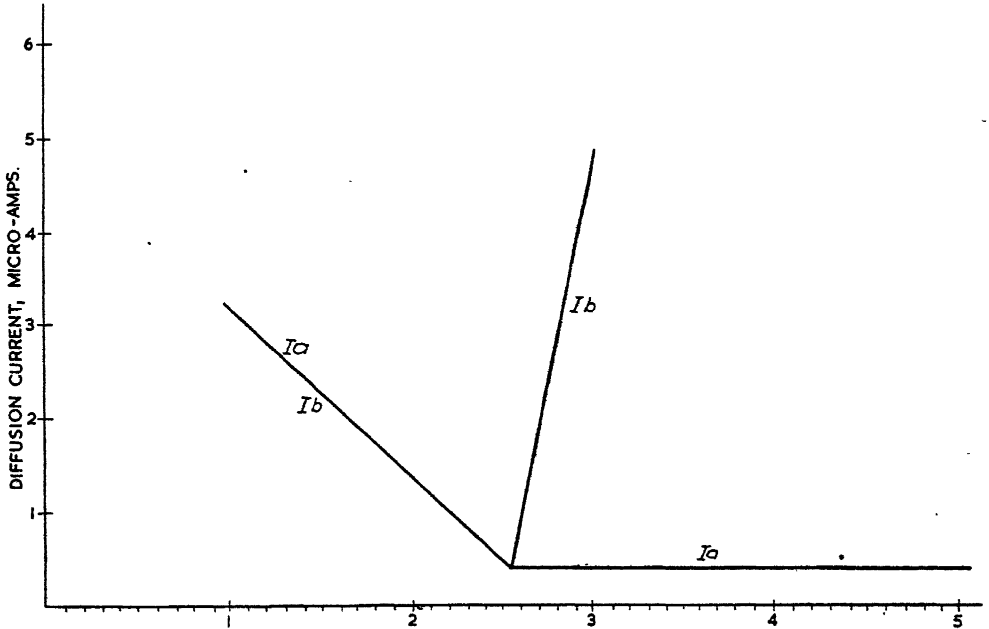

Fig. 1. Titration of 25 ml. of 0·001 *M* copper in 0·5 *M* citrate buffer (pH = 4) with 0·1964 *M* cupferron solution
(*a*) E_{app} = —0·5 volt
(*b*) E_{app} = —1·45 volt

As was anticipated, the current at the end-point at this more negative potential is much greater than when the titration is carried out at —0·5 volt.

Table I summarises some results obtained in the titration of copper under various conditions. In all instances a volume of 25 ml. of the copper - buffer - gelatin mixture was titrated.

It is seen that solutions of copper with a concentration between 5×10^{-4} and 2×10^{-3} *M* can be titrated by the recommended procedure with an accuracy and a precision of 1 per cent. or better.

Unsatisfactory results are obtained in unbuffered solutions that contain 0·01 *N* or more hydrochloric acid. The precipitation of copper cupferronate becomes slower, its solubility increases and there is opportunity for decomposition of cupferron at this low pH before it has reacted with copper. This explains the high results obtained in the first two experiments of Table I. Oxalate is unsuitable as a buffer, because the precipitation is extremely slow and incomplete at a pH of the order of 3. No precipitation was obtained from 0·1 *M* ammonia, or ammonium tartrate - ammonia mixtures.

Experiments have been carried out in 0·3 *M* citrate buffers. Although satisfactory results were obtained, large concentrations of these anions which form complexes with copper should be avoided, because the solubility of the precipitate increases and the precipitation becomes slower. A high concentration of potassium hydrogen fluoride in the buffer did not affect the titration (Table I). Use of this fact is made in the titration of copper in presence of much ferric iron.

An attempt has been made to titrate univalent copper with cupferron. The cuprous copper solution was prepared by reduction of cupric copper with hydroxylamine hydrochloride in presence of a citrate buffer. No precipitate was formed upon addition of cupferron, but a white precipitate separated after addition of a large excess of reagent.

TABLE I

AMPEROMETRIC TITRATION OF 25 ML. OF COPPER SOLUTION WITH CUPFERRON UNDER VARIOUS CONDITIONS

Buffer medium	pH	Concentration of copper, molarity	Copper present, mg.	Copper found, mg.	Error %
0·1 *N* HCl	1	10^{-3}	1·589	(2·4)	+ 50
0·01 *N* HCl + 0·09 *N* KCl..	2	10^{-3}	1·589	1·698	+ 6·8
Biphthalate (0·05 *M*) ..	3	$1·5 \times 10^{-3}$	2·384	2·374	− 0·4
” ..	5·2	5×10^{-4}	0·795	0·797	+ 0·2
” ..	5·2	10^{-3}	1·589	1·589	0·0
” ..	5·2	2×10^{-3}	3·178	3·147	− 1·0
Citrate (0·05 *M*)	4	10^{-3}	1·589	1·592	+ 0·2
”	5·11	10^{-3}	1·589	1·580	− 0·6
”	6	10^{-3}	1·589	1·602	+ 0·8
Citrate + 0·5 *M* KHF_2 ..	4	10^{-3}	1·589	1·602	+ 0·8
Tartrate (0·05 *M*)	4	10^{-3}	1·589	1·604	+ 0·9
Acetate (0·05 *M*)	4·78	10^{-3}	1·589	1·580	− 0·6
Oxalate (0·05 *M*)	2·5*a*	10^{-3}			
	3·5*a*	10^{-3}			

a Precipitation occurs only after addition of excess of reagent and standing.

TITRATION OF FERRIC IRON

Ferric cupferronate is considerably less soluble than the corresponding copper compound. For this reason, the iron titration can be carried out at a lower pH than that of copper. In the procedure given below the titration cell is covered with a coat of black paint or wrapped in black paper to protect the solution from light. In agreement with an observation by Lingane[8] we found that ferric iron in the form of a complex with tartrate or nitrate is readily reduced upon exposure to light.

Procedure—Place a measured volume of the iron solution in the (blackened) titration cell and add a citrate or tartrate buffer with a pH between 3 and 4. Make the final concentration of the buffer 0·05 *M* or less. Add gelatin to give a concentration of 0·01 per cent.; remove oxygen, titrate with 0·03 *M* cupferron solution and proceed further as described in the procedure for copper. The end-point corresponds to the formation of $Fe(cupf)_3$.

Discussion—Some experimental results are summarised in Table II. It is seen that according to the recommended procedure the accuracy is of the order of 1 per cent. when the iron concentration is 0·001 *M* or greater. When the iron concentration is less the diffusion current of the iron becomes very small (the diffusion coefficient of the complex is small and the reduction involves only one electron), and consequently the relative error becomes greater. The pH of the buffer used should not be greater than 5, because at greater pH values the precipitation becomes incomplete, the composition of the precipitate changes and low results are obtained.

Oxalate, as in the titration of copper, is unsuitable as a buffer. No precipitation was obtained from ammoniacal tartrate solutions.

The titration can be carried out in 0·1 to 0·01 *N* hydrochloric acid (at a potential of −0·3 volt) with the same accuracy as in the recommended buffers. However, in this relatively strong acid medium it is difficult to coagulate the bulky precipitate, which makes the current measurement more difficult. Several titrations have been carried out in 0·1 *N* hydrochloric acid at a potential of −1·2 volts at which potential the diffusion current of cupferron is also measured, but this is not recommended, for reasons mentioned above. The accuracy of the titration of 0·001 *M* ferric iron was found not to be affected by the presence of a fifty-fold excess (molar) of aluminum, zinc, chromium^{III}, manganese and cadmium.

The titration of ferrous iron in a citrate buffer (pH 4 to 6) was investigated by measuring the anodic diffusion current of ferrous iron. The results were unsatisfactory because of incomplete precipitation of the yellow ferrous cupferronate.

TABLE II

AMPEROMETRIC TITRATION OF 25 ML. OF FERRIC IRON SOLUTION WITH CUPFERRON UNDER VARIOUS CONDITIONS

Buffer medium	pH	Concentration of iron, molarity	Iron present, mg.	Iron found, mg.	Error %
0·1 *N* HCl	1	$1{\cdot}04 \times 10^{-3}$	1·453	1·441	− 0·8
"	1	$2{\cdot}082 \times 10^{-3}$	2·907	2·871	− 1·2
"	1	$5{\cdot}205 \times 10^{-4}$	0·727	0·742	+ 2·0
0·01 *N* HCl	2	$2{\cdot}082 \times 10^{-3}$	2·907	2·874	− 1·2
"	2	$1{\cdot}041 \times 10^{-3}$	1·453	1·438	− 1·0
"	2	$5{\cdot}205 \times 10^{-4}$	0·727	0·723	− 0·9
Citrate (0·05 *M*)	3	$1{\cdot}041 \times 10^{-3}$	1·453	1·463	− 0·7
"	3	$5{\cdot}205 \times 10^{-4}$	0·727	0·746	+ 2·6
"	4·4	$2{\cdot}082 \times 10^{-3}$	2·907	2·879	− 1·0
"	4·4	$1{\cdot}041 \times 10^{-3}$	1·453	1·452	0·0
"	5·1	$1{\cdot}041 \times 10^{-3}$	1·453	1·463	− 0·7
"	6·0	$1{\cdot}041 \times 10^{-3}$	1·453	1·327	− 6·4
"	6·3	$1{\cdot}041 \times 10^{-3}$	1·453	1·284	− 11·5
Tartrate (0·05 *M*)	4·0	$2{\cdot}082 \times 10^{-3}$	2·907	2·934	+ 0·9
"	4·0	$1{\cdot}041 \times 10^{-3}$	1·453	1·467	+ 0·9
"	5·8	$2{\cdot}082 \times 10^{-3}$	2·907	2·720	− 6·5
Oxalate (0·05 *M*)	2·5*a*	$1{\cdot}041 \times 10^{-3}$			
	3·5*a*	$1{\cdot}041 \times 10^{-3}$			

a Precipitation occurs only after addition of excess of reagent.

MIXTURE OF CUPRIC COPPER AND FERRIC IRON

According to Pinkus and Martin[10] the solubilities of copper and ferric cupferronates are $1{\cdot}1 \times 10^{-5}$ and 3×10^{-7} *M* respectively. It may be expected that upon titration of a mixture of copper and iron in a citrate or tartrate buffer the iron is precipitated first, provided that the stabilities of the iron and copper complexes are of the same order of magnitude. The slope of the precipitation line of copper in a citrate or tartrate buffer is considerably steeper than that of an equimolar solution of iron, because (1) the reduction of copper involves two electrons and that of iron one and (2) one mole of copper reacts with two moles of cupferron, and one mole of iron with three moles. Twenty-five ml. of a mixture that was $1{\cdot}041 \times 10^{-3}$ *M* in respect of ferric iron and 0·001 *M* in respect of copper was titrated in a citrate buffer of pH 5 with 0·02 *M* cupferron solution. The titration lines are given in Fig. 2.

By extrapolation of the straight portions of the first (iron) and second (copper) precipitation lines the iron end-point was found to correspond to an over-consumption of iron, the error being of the order of +8 per cent. The method is not suitable for the separate titration of iron and copper in a mixture but the sum of copper and iron is found accurately to within about 2 per cent. Fig. 2 shows conclusively that first iron is precipitated, then follows a region in which both iron and copper are precipitated and finally copper cupferronate precipitates alone. Fig. 2 also illustrates clearly that the precipitation line of copper is considerably steeper than that of iron.

It had been found that iron can be titrated quantitatively in 0·1 *N* hydrochloric acid (Table II), whilst copper precipitates in this medium only upon addition of an excess of reagent. Upon titration of an equimolar (0·001 *M*) mixture of copper and iron in 0·1 *N* hydrochloric acid iron was found to precipitate first quantitatively; at the end-point the diffusion current of copper is measured. Upon careful further addition of reagent this current does not change until the excess is large enough to initiate the precipitation of copper cupferronate. In mixtures in which the ratio of ferric iron to copper was one or greater than one the iron could be titrated with an accuracy of 1 per cent. When copper is in excess of iron the accuracy becomes less.

A procedure was developed for the titration of copper in presence of a large excess of iron by making the iron harmless in the form of a fluoride complex. Upon addition of a large excess of potassium hydrogen fluoride to a mixture of copper and iron in a citrate buffer (0·05 *M*; pH 5) most of the iron precipitates in the form of K_3FeF_6. The dissolved iron does not interfere with the titration. As shown in Table I, the titration of copper is not affected by a large excess of fluoride.

Procedure—To the mixture of copper and iron in a citrate buffer (0·05 *M*, pH 5) add $KHF_2.2H_2O$ to give a concentration of 0·5 *M*. Proceed further as described in the procedure for copper.

Discussion—Some results are given in Table III. It is seen that the accuracy of the copper titration is not affected by a twenty-five fold molar excess of ferric iron.

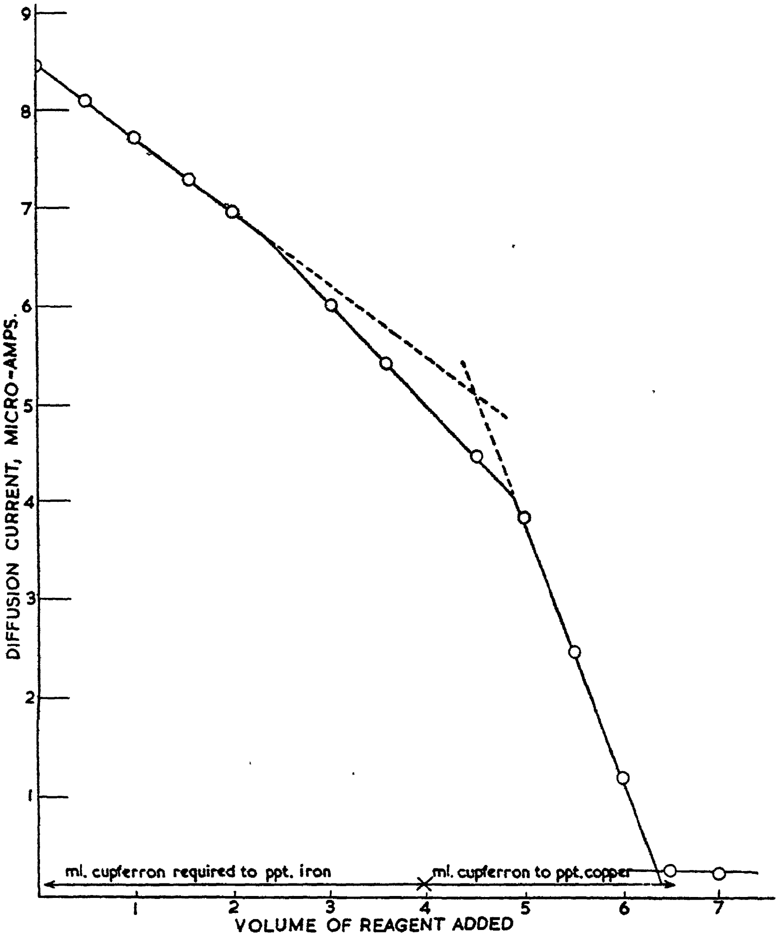

Fig. 2. Titration of 25 ml. of solution 10^{-3} *M* in respect of copper and $1\cdot041 \times 10^{3}$ in respect of ferric iron in citrate buffer (pH = 5·1) at —0·5 volt

TABLE III

TITRATION OF 25 ML. OF 0·001 *M* COPPER SOLUTION IN PRESENCE OF IRON AND BIFLUORIDE (see Procedure)

pH	Concentration of iron^{III}	Ratio copper^{II}/iron^{III}	Copper present,	Copper found,	Error
	molarity		mg.	mg.	%
5·1	0·001	1	1·59	1·58	— 0·6
4·1	0·0005	0·5	1·59	1·602	+ 0·7
4	0·01	10	1·59	1·61	+ 1·2
5	0·025	25	1·59	1·594	+ 0·2
5·5	0·005	5	1·59	1·595	+ 0·3

SUMMARY

1. Cupferron solutions prepared in conductivity water and kept in dark bottles are stable for at least fourteen days.

2. Procedures are given for the amperometric titration of copper and of ferric iron in suitable buffer solutions, with cupferron. With 0·001 *M* solutions of the metals the accuracy and precision of the method is 1 per cent. The accuracy of the iron titration is not affected by a fifty-fold excess of aluminium, zinc, chromium^{III}, manganese and cadmium.

3. When the ratio of iron to copper is equal to or greater than one, the iron can be titrated in a medium of 0·1 *N* hydrochloric acid. The end-point is found before the copper precipitates.

4. Copper can be titrated in presence of a large excess of iron in a citrate buffer that is 0·5 *M* in respect of potassium hydrogen fluoride.

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SCHOOL OF CHEMISTRY
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Determination of Small Quantities of Aluminium in Gunmetal*

BY J. B. PRICE AND S. T. PAYNE

IT is well known that small amounts of aluminium give rise to undesirable properties in cast bronzes and gunmetals and this paper describes a method for the accurate determination of aluminium in such alloys, with particular reference to contents in the range 0·001–0·01 per cent.

Briefly, the method involves separation of the aluminium from copper, tin, zinc, etc., by mercury cathode electrolysis,[1] followed by determination of the aluminium, using aluminon and measuring the resulting colour on a Spekker absorptiometer.[2]

The recommended method is described in detail in the first section of the paper and the second section gives some experimental work carried out in the development of the method.

I. METHOD

REAGENTS—

Buffer solution—Dissolve 156 g. of ammonium acetate and 108 g. of ammonium chloride in 800 ml. of water and filter if necessary. To the solution add 200 ml. of freshly prepared 5 per cent. gum arabic solution made by dissolving selected gum arabic "crystals" in hot water and filtering. The combined solution will keep almost indefinitely.

Aluminon (*Aurintricarboxylate*) *solution*—Prepare a 0·2 per cent. aqueous solution of aluminon as described by J. A. Sherrer and W. H. Smith.[3] This must be allowed to "age" for 3 to 4 days before use.

Ammonium borate solution—Dissolve 93 g. of boric acid in 1 litre of *N* ammonia and dilute to 0·8 *N* (with respect to titratable ammonium ion), standardising with *N* hydrochloric acid and using screened methyl orange indicator.

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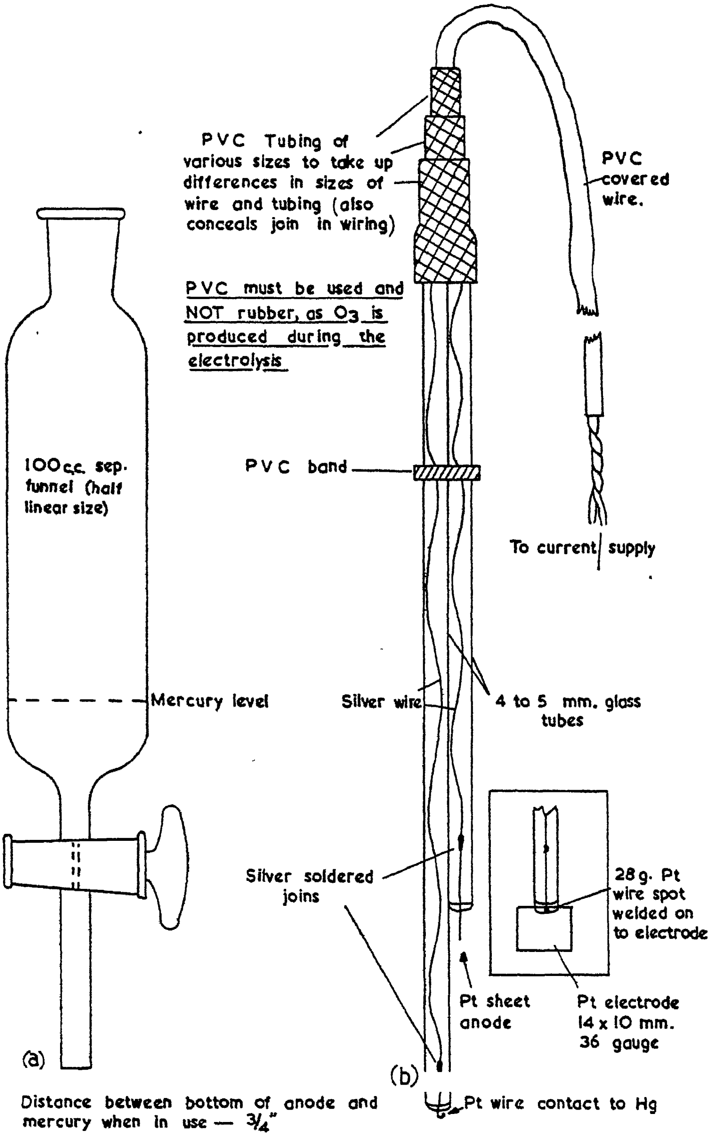
* A communication from the British Non-Ferrous Metals Research Association.

PROCEDURE—

Weigh out a 0·5 g. sample into a 450 ml. conical wide mouth beaker, dissolve in 7·5 ml. of 20 per cent. sulphuric acid plus 1·5 ml. of concentrated nitric acid and when the reaction has ceased, evaporate to fuming of sulphur trioxide and continue fuming until all the nitric acid has been completely removed. Cool, take up in 25 ml. of water, heat to boiling, cool and dilute to 50 ml. Transfer to a mercury electrolysis cell (Fig. 1 *a*) containing about 15 ml. of clean mercury, insert electrodes (Fig. 1 *b*), mark the liquid level on the cell and electrolyse for not less than 20 hours at 1 amp. The loss of liquid by electrolytic breakdown may be considerable and the solution should be made up to the mark with distilled water just before the electrolysis is due to be discontinued. When the electrolysis is completed, run off the mercury and a little of the solution and then pipette off 20 ml. of the remaining solution and proceed according to (A) or (B).


PVC Tubing of various sizes to take up differences in sizes of wire and tubing (also conceals join in wiring)
PVC covered wire.
PVC must be used and NOT rubber, as O3 is produced during the electrolysis
100c.c. sep. funnel (half linear size)
PVC band
To current supply
Mercury level
Silver wire
4 to 5 mm. glass tubes
28g. Pt wire spot welded on to electrode
Silver soldered joins
Pt sheet anode
Pt electrode 14 x 10 mm. 36 gauge
(a)
(b)
Distance between bottom of anode and mercury when in use — 3/4"
Pt wire contact to Hg

Scale of figure, half actual size throughout

Fig. 1. Mercury cathode electrolysis cell (Pyrex glass)

(A) *Adjustment of pH by means of pH meter*—Transfer the aliquot to a 50 ml.-squat beaker, add 5 ml. of the buffer solution and then adjust to a pH of 4·7 ± 0·05 with diluted aqueous ammonia (1 in 4), using the glass electrode. Transfer the solution to a 50-ml. graduated flask and proceed according to (C).

(B) *Adjustment of pH by means of indicator*—As an alternative to (A) transfer the aliquot to a 50-ml. graduated flask and add 1 drop of methyl red solution followed by diluted aqueous ammonia (1 + 1) until *just* yellow. Then adjust carefully to the red colour with *N* hydrochloric acid, replace the stopper in the flask and shake well. The colour may change back to yellow owing to ammonia vapour in the neck of the flask, in which event more acid is added until the red colour just reappears. Add 3·5 ml. of *N* hydrochloric acid in excess. *This should be accurately added from a burette.* Next add 2 drops of bromine water and allow to stand for 1 minute, then add 4 drops of 10 per cent. hydroxylamine hydrochloride solution followed by 5 ml. of the buffer solution. Replace the stopper, shake well and proceed according to (C).

(C) After adjusting the pH by method (A) or (B) add 2 ml. of the aluminon solution and mix. Place the flask in a boiling water bath and leave for 10 min. (± 1 minute). Cool the solution, add 4 ml. of the ammonium borate solution and finally dilute to the mark and mix well. Allow to stand for 5 minutes after introducing the borate and then measure the absorption on the Spekker, using the mercury lamp, H.503 and Ilford 604 filters, and 4 cm. cells. Replace the solution by water and measure the absorption again to obtain the cell blank. The difference between the two readings is referred to the calibration graph and the percentage of aluminium obtained. A blank determination made on the original acids, without metal and treated throughout as an ordinary sample, should be carried out with each batch, and the percentage of aluminium thus indicated deducted from that found in the alloy.

The method is suitable for an aluminium range from 0·001 to 0·01 per cent. in the sample. Higher percentages can be dealt with by taking a smaller aliquot and/or a smaller weight of sample and/or a smaller Spekker cell and calibrating in the appropriate range. The final solution in which the colour is developed should not contain more than 80 μg. of aluminium.

CALIBRATION—

Standard aluminium solution—Dissolve 0·1 g. of super-purity aluminium in 20 per cent. sulphuric acid and dilute to 1 litre with 10 per cent. acid; dilute 100 ml. of this solution further to 1 litre with 10 per cent. acid. This solution contains 10 μg. of aluminium per ml.

Standards for samples containing from 0 *to* 0·01 *per cent. of aluminium*—Into five 50-ml. graduated flasks (A to E) introduce quantities of the standard aluminium solution as follows:—

Flask	A	B	C	D	E
Standard solution, ml. ..	nil	0·25	0·50	0·75	1·00
≡ Al in sample, % ..	nil	0·0025	0·0050	0·0075	0·0100

To each flask add 20 per cent. sulphuric acid as follows:—

20% sulphuric acid, ml. ..	3·0	2·8	2·7	2·6	2·5

Finally add 17 ml. water to each; then treat as an aliquot from an ordinary sample after electrolysis. Measure the absorption, using a 4-cm. cell. Deduct the reading for A from each of the others and plot the difference against the percentage of aluminium.

Standards for samples containing from 0·010 *to* 0·040 *per cent. of aluminium*—Repeat as above, using quantities of standard solution as follows:—

Flask	A	B	C	D	E
Standard solution, ml. ..	nil	2	4	6	8
≡ Al in sample, % ..	nil	0·010	0·020	0·030	0·040

To each flask add 20 per cent. sulphuric acid as follows:—

20% sulphuric acid, ml. ..	4·0	3·0	2·0	1·0	nil

Finally add 16 ml. water to each, then proceed as for an aliquot from an ordinary sample. Measure the absorption, using a 1-cm. cell.

Deduct the reading for A from each of the others and plot the differences against the percentage of aluminium.

II. EXPERIMENTAL

(a) MERCURY CATHODE ELECTROLYSIS—

(i) *Removal of iron*—It is well known that iron, like aluminium, produces a colour with aluminon, and experiment showed that 0·02 per cent. of iron is equivalent to 0·001 per cent. of aluminium. If the conditions of electrolysis set out in the above method are followed, the removal of iron is sufficient to avoid interference.

It was, however, thought advisable to formulate a test by which the removal of iron could be shown to be efficient: it was found that a solution containing the equivalent of 0·01 per cent. of iron gave a pale but definite pink colour with a solution of potassium thiocyanate, whereas the solution obtained after the prescribed electrolysis gave no colour. If therefore a 20-ml. aliquot is taken after electrolysis, treated with 0·25 ml. of nitric acid and diluted to 40 ml., and then 10 ml. of 10 per cent. potassium thiocyanate solution are added, the appearance of a pink colour is sufficient to show that the determination should be discarded if an accurate result is desired.

(ii) *Interference of phosphorus*—It was noticed that certain samples remained cloudy after electrolysis for the prescribed time, and in the first instance tin was suspected of being present in a different and more resistant form. However, on centrifuging off this precipitate after 20 hours electrolysis and examining its character, it was found to be tin phosphate. In no instance where phosphorus was absent was any precipitate left after 20 hours electrolysis. Further, the phosphorus when present combines completely with metastannic acid, provided the latter is in excess.

It was found that electrolysis for several days was necessary to remove all the tin phosphate from an alloy containing 0·5 per cent. of phosphorus and it was proposed therefore to determine if loss of aluminium occurred when the tin phosphate was centrifuged off after the normal electrolysis time of 20 hours. Four 0·5-g. samples of pure bronze (90 per cent. Cu, 10 per cent. Sn) used in previous experiments were dissolved; to each of the resulting solutions an addition of aluminium equivalent to 0·005 per cent. was made and, to two only, the equivalent of 0·5 per cent. of phosphorus was also added. These samples were all electrolysed with mercury cathodes for 20 hours, at the end of which time those without phosphorus were quite clear, whilst the others remained cloudy. The two latter were transferred to centrifuge tubes and rotated at 4,000 r.p.m. (6-inch radius) for 15 minutes and the clear liquids poured off. All four samples were then treated with aluminon and the aluminium determined, with the following results:—

	Aluminium added %	Phosphorus added %	Aluminium found %
1.	0·005	nil	0·0053
2.	0·005	nil	0·0056
3.	0·005	0·5	0·0047
4.	0·005	0·5	0·0049

It would appear, therefore, that for alloys containing 0·5 per cent. of phosphorus, the removal of tin phosphate by centrifuging at the end of the usual period of electrolysis causes a small loss of aluminium equivalent to less than 0·0005 per cent.

(b) FORMATION OF ALUMINON COMPLEX—

A very thorough testing of the method of Strafford and Wyatt for aluminium in water[2] was carried out and it was found that the conditions laid down were suitable for adaptation to the determination of aluminium in alloys, but it was decided that, owing to the somewhat temperamental nature of aluminon the conditions of the method would have to be adhered to strictly if errors were to be avoided.

However, the method of heating adopted by Strafford and Wyatt for developing the colour, *i.e.*, by boiling on the hot plate, suggested the possibility of some loss of acid and consequent change in pH which would vary according to the rate at which boiling took place. This was confirmed experimentally, and to overcome the difficulty the solutions in which the colour was being developed were placed in flasks in a vigorously boiling water-bath for 10 minutes. During the heating, glass stoppers were loosely placed in position and under these conditions it was found that the pH remained extremely constant, measurements being taken after cooling the solutions in running water.

sodium hydroxide solution contributing an apparent 0·85 μg. of mercury, but this blank error becomes constant after the insoluble matter in the sodium hydroxide solution has settled. A. O. Jones

Colorimetric Determination of Morphine. J. S. N. Cramer and J. G. Voerman (*Pharm. Weekblad*, 1949, **84**, 129–133)—In the reaction of Guarino (*Boll. Soc. ital. Biol. sper*, 1946, **22**, 1231) a colour is obtained by the action of iodine and ferric chloride on morphine. This is not very satisfactory for colorimetric purposes, but can be greatly improved by replacing the iron by nickel.

Procedure—Take a quantity of aqueous morphine solution or extract, containing not more than 8 mg. of morphine, in a 50-ml. measuring flask, dilute with water to 15 ml., and add, in succession, 15 ml. of 0·1 N hydrochloric acid and 2 ml. of 5 per cent. iodic acid solution. After 2 min., add 5 ml. of a saturated solution of ammonium carbonate, and dilute to the mark with a 5 per cent. solution of ammonium carbonate. After 30 min., add 1 ml. of 1 per cent. nickel sulphate solution, and set aside for 90 min. Prepare a blank at the same time with water substituted for the iodic acid. Determine the extinction (Klett Summerson photo-electric colorimeter, red filter). With this reaction, no colour is given by the five other chief alkaloids of opium, or by meconic acid. G. Middleton

Detection and Determination of Cocaine in the Presence of Procaine and Various Other Substances. J. L. Young (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 781–783)—Cocaine is the methyl ester of benzoyl ecgonine and when subjected to mild alkaline hydrolysis yields methyl alcohol, which can be determined by Beyer's modification of the Georgia-Morales test. Thus, in mixtures, only compounds that yield methyl alcohol on hydrolysis will interfere. Mixtures of cocaine with procaine, other synthetic drugs, as well as opium alkaloids and such diluents as lactose, starch, and sucrose were hydrolysed and the presence of these substances did not affect the test. The sensitivity is approximately 2 mg. of cocaine hydrochloride.

METHOD—*Reagents*—*Potassium permanganate solution*—Dissolve 3 g. of potassium permanganate and 15 ml. of 85 per cent. phosphoric acid in water and dilute to 100 ml. *Oxalic acid reagent*—Dissolve 5 g. of oxalic acid in 100 ml. of diluted sulphuric acid (1 : 1). *Modified Schiff's reagent*—Dissolve 0·2 g. of rosaniline hydrochloride in 120 ml. of hot water, cool, and add 2 g. of sodium hydrogen sulphite previously dissolved in 20 ml. of water. Add 2 ml. of hydrochloric acid, dilute the solution to 200 ml., and place in a refrigerator for 24 hr. before use. Store in a cool place. *Standard cocaine solution*—Dissolve 50 mg. of pure cocaine hydrochloride in 10 ml. of water; 0·1 ml. of this solution $\equiv$ 0·5 mg. of cocaine hydrochloride.

Qualitative procedure—Introduce 25 to 50 mg. of the alcohol-free sample into a small distilling flask, and add 2 ml. of 2 per cent. aqueous sodium hydroxide solution and 5 ml. of water. Connect the flask to a condenser with a ground-glass joint and distil the contents until 2 ml. of distillate are collected in a glass-stoppered cylinder. Add 0·25 ml. of 24 per cent. ethyl alcohol and 2·75 ml. of water. Add 2 ml. of the potassium permanganate solution and allow to stand, with occasional shaking, for 10 min. Add 2 ml. of the oxalic acid reagent and 5 ml. of Schiff's reagent, tightly stopper the cylinder, and invert three times. Examine after 1 and 2 hr. A blue or violet colour indicates the presence of cocaine in the sample.

Quantitative procedure—Weigh 25 mg. of sample, continue as in the qualitative procedure, but collect the 2-ml. distillate in a previously calibrated Nessler tube. Introduce 0·4-, 0·6-, and 1·0-ml. portions of the standard cocaine solution into separate distilling flasks and collect 2-ml. distillates in Nessler tubes. Add reagents, as in the qualitative procedure, to the test and standard distillates and allow the Nessler tubes to stand for 2 hr. Compare the colour developed by the sample with those of the primary standards. To obtain an accurate determination make a final comparison with a series of standards, in 0·1-ml. increments, in the region of the nearest primary standard.

A permanent standard curve may also be prepared from extinction readings at 560 mμ. obtained by the use of suitable photometric apparatus.

A. H. A. Abbott

Report on Oils, Fats, and Waxes. J. Fitelson (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 358–359)—The chromatographic technique for purification of unsaponifiable matter (Sylvester *et al.*, *Analyst*, 1945, **70**, 295) has been studied as an alternative to the official S.P.A. washing procedure (*Methods of Analysis of the A.O.A.C.*, 31.40; *Analyst*, 1933, **58**, 207) and, as the results showed very close agreement, further collaborative studies are planned.

Preliminary study of the application of the tentative modified Bellier test (*A.O.A.C.*, 31.47–31.48) to mixtures of peanut oil with other vegetable oils indicates an approximately quantitative relation between the turbidity temperature and the peanut oil content, and further investigation is planned.

Studies of methods for determination of antioxidants in oils are in progress.

It is recommended that the official S.P.A. method for unsaponifiable matter (*loc. cit.*) be made official as well as the method for squalene in oils (*Methods of Analysis of the A.O.A.C.*, 31.41–31.43).

A. O. Jones

Biochemical

New Colour Reactions for Steroids. M. Pesez (*Bull. Soc. Chim.*, 1947, 911–914M)—There are no specific reactions for the tetracyclic steroid derivatives, but the reactions described are characteristic for certain functions of these compounds.

Δ^4-*Androstene*-17-*ol*-3-*one*(cis), cis-*testosterone*,—m.p. 220·5° C., α_D + 72° (ethanol, 1 per cent.). *Glyoxylic reaction*—*cis*-Testosterone dissolved in 2 ml. of a mixture of 1 volume of 96 per cent. ethanol and 2 volumes of sulphuric acid gives a yellow colour with a greenish-yellow fluorescence.

On adding 0·5 ml. of glyoxylic reagent (5 per cent. oxalic acid solution reduced with zinc - copper couple) without mixing, a red-violet ring is formed and, on mixing, the colour of the solution becomes red-violet with a red fluorescence. The red colour disappears on dilution with water, but persists in alcoholic sulphuric acid. Acetic and propionic esters give the same reaction. The limit of sensitivity is 1 to 2 μg. *trans*-Testosterone gives neither the colour nor the fluorescence. A strongly dichromatic red-blue colour reaction and a red fluorescence are produced if the glyoxylic reagent is replaced by a 2 per cent. aqueous or alcoholic solution of acetaldehyde, acetone, or methylethyl ketone.

Reaction with cupric and ferric salts—Dissolve *cis*-testosterone in 2 ml. of a copper reagent made by mixing equal parts of sulphuric acid and a 5 per cent. solution of copper sulphate, and heating over boiling water. A Prussian blue colour develops, but disappears on dilution with water. The reaction is characteristic for the *cis*-isomer and is sensitive to 5 μg. of steroid. *cis*-Testosterone does not react in the cold with a mixture of 3 volumes of sulphuric acid and 2 volumes of 5 per cent. ferric sulphate solution, but on warming at 40° C., a green colour develops, and changes to blue at 70° C., and to yellow at 100° C. The reaction is sensitive to 10 μg. of the *cis*-isomer and is not given by the *trans*-form. The colour at 40° to 50° C. can be used for the colorimetric estimation of 0·1 to 1·0 mg. of *cis*-testosterone.

Δ^4-*Androstene*-17-*ol*-3-*one*(trans), trans-*testosterone*, —m.p. 154° C., $\alpha_D + 111°$ (ethanol, 1 per cent.), Δ^4-*androstene*-3:17-*dione*—m.p. 171 °C., $\alpha_D + 190°$ (ethanol, 1 per cent.). The reactions with glyoxylic reagent and copper and iron salts described above are negative with these compounds, but become positive after heating with alcoholic sulphuric acid. Dissolve the substances without heating in 2 ml. of alcoholic sulphuric acid: no appreciable colour or fluorescence is produced. Warm for a few minutes over boiling water, cool, and add 0·5 ml. of glyoxylic reagent, whereupon an intense red colour is produced. The limit of sensitivity of the reaction is 2 μg. for androstenedione and 5 μg. for *trans*-testosterone. The reaction is negative with androstane-3:17-dione.

The copper and iron reactions are produced in the same way by warming the substance with 2 ml. of alcoholic sulphuric acid. After cooling, add 0·5 ml. of a 10 per cent. solution of cupric sulphate or 5 per cent. ferric sulphate; after heating to 50° to 70° C. a blue-green colour develops. 0·1 to 1·0 mg. of *trans*-testosterone and its esters can be estimated by the ferric sulphate reaction. The esters in solution in oil can be estimated after cold saponification and extraction with ether.

Δ^5-*Androstene*-3-*ol*(trans)-17-*one*, trans-*dehydroandrosterone*—m.p. 151° C. (slow heating), $\alpha_D + 11{\cdot}5°$ (ethanol, 1 per cent.). To prepare the reagent mix equal parts of a 10 per cent. solution of ferric alum and sulphuric acid and cool. The solution has a slight yellow colour and it should be discarded if, after a time, the colour becomes troublesome. To 2 ml. of reagent add *trans*-dehydroandrosterone or an ester thereof. Heat the mixture at 38° to 40° C., whereupon a rose colour that changes to an intense red-violet is produced. The colour can be used for the estimation of 100 to 500 μg. of material. The limit of sensitivity is 5 μg. The reaction is characteristic for *trans*-dehydroandrosterone, and is negative with the following steroids: androsterone, cholesterol, cholestanol, cholestanone, norcholestenolone, norcholestenedione, oxycholenic acid, hydroxy-aetio-cholenic acid, Δ^4-androstene-3 : 17-dione, *trans*-testosterone, Δ^5-pregnene-3-ol-20-one, progesterone, folliculine and related oestrogens. The colours obtained with *cis*-testosterone and its esters, methyl-testosterone, and Δ^5-androstene-*trans*-3-*trans*-17-diol are blue-green, salmon-red, and brownish-red, respectively. The reaction with androstene-diol is feeble because of the insolubility of the product in hydrated sulphuric acid. It becomes positive on dissolving the steroid in 2 ml. of benzene and stirring with 2 ml. of ferric sulphate reagent, when a red-violet colour appears at 38° to 40° C. This reaction is negative with the esters of Δ^5-androstene-*trans*-3-*trans*-17-diol, -3-acetate, -17-benzoate, and -3-acetate-17-benzoate. For this reaction the iron alum cannot be replaced by other salts.

Keto-steroids—o-*Nitrobenzaldehyde reaction*—To 5 ml. of an alcoholic solution of keto-steroid, add 0·5 ml. of a 0·5 per cent. solution of *o*-nitrobenzaldehyde in absolute alcohol and 0·5 ml. of 10 per cent. sodium hydroxide solution, heat over boiling water for 1 min., and add 5 ml. of water, whereupon a very stable greenish-yellow colour develops. With the 17-keto-steroids Δ^4-androstene-3 : 17-dione, *trans*-dehydro-androsterone, equiline, and folliculine, the colour is more intense than with the 3-keto-steroids: progesterone, testosterone, and deoxycorticosterone. The reaction is negative with the 20-keto-steroids and the non-ketonic steroids. This reaction can be used for the colorimetric estimation of folliculine and *trans*-dehydro-androsterone in amounts varying from 0·2 to 2·0 mg.

m-Nitrobenzene - pyridine reaction—The reagent is a 0·5 per cent. solution of *m*-dinitrobenzene (free from the *ortho*-derivative) in pyridine that has been distilled over potassium (b.p. 116° to 117° C.). To a sample of keto-steroid dissolved in 2 ml. of the reagent, add 2 ml. of water and, after mixing, 0·4 ml. of 10 per cent. sodium hydroxide solution. Stir thoroughly, whereupon a violet colour slowly develops; this changes to red and reaches maximum intensity in 2 hr., but changes slowly to brown in 6 hr. The colour is stable for several hours with the 17-keto-steroids Δ^4-androstene-3:17-dione, dehydro-androsterone, equilenine, folliculine, equiline, androsterone, and androstane-3-ol-17-one (*iso*-androsterone). A feeble positive reaction is given by the unsaturated 3-keto-steroids progesterone, testosterone, methyl-testosterone, ethinyl-testosterone, norcholestene-dione, deoxycorticosterone acetate. On the contrary, the saturated 3-keto-steroids cholestane-3-one and androstane-17-ol-3-one, give a strong positive reaction. The reaction is negative with the non-keto, and 20-24-ketosteroids cholesterol, cholestanol, norcholestene-3-ol-24-one, hydroxy- and hydroxy-aetio-cholenic acids, Δ^5-pregnene-3-ol-20-one, Δ^5-acetoxy-pregnene-3-ol-20-one, androstane-3 : 17-diol, Δ^5-androstene-3 : 17-diol, $\Delta^{1,4}$-androstadiene-17-ol-3-one, dihydrofolliculine, and cholic acid.

Below is a summary of the new colour reactions:

Steroids	Reactions positive
cis-Testosterone	Glyoxyl, cupric, ferric (all direct)
trans-Testosterone, Δ^4-Androstene-3 : 17-dione	Glyoxyl, cupric, ferric (all after heating with alcoholic sulphuric acid)
trans-Dehydroandrosterone	Ferric alum
17-Keto-steroids, 3-Keto-steroids	*o*-Nitrobenzaldehyde, *m*-dinitrobenzene - pyridine (unsaturated 3-keto-steroids give weak reactions with the latter)

J. S. Harrison

Agricultural

Determination of Total Boron in Plant Material with "Chromotrope-B." C. M. Austin and J. S. McHargue (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 427–431)—The method presented is free from many of the disadvantages of the official quinalizarin method (*Methods of Analysis of the A.O.A.C.*, 6th Ed.) and of other methods. The reagent is a 0·0005 per cent. solution of *p*-nitrobenzeneazo-1 : 8-dihydroxynaphthalene-3 : 6-disulphonic acid ("Chromotrope-B") in 95·5 per cent. sulphuric acid.

Preparation of the standard curve—Dissolve 0·0930 g. of anhydrous sodium pyroborate in several hundred ml. of 70 per cent. acetic acid, add 100 g. of re-crystallised barium hydroxide octohydrate and, when dissolution is complete, dilute the liquid to 1 litre. This solution contains 20 μg. of boron per ml. Prepare from it solutions containing up to 15 μg. of boron per ml., treat 1 ml. of each solution as described (*infra*) and determine the percentage transmission at a convenient and reproducible temperature between 20° and 30° C., and from these data construct a curve relating concentration to transmission.

Procedure—To 0·2 to 0·5 g. of air-dried, 60-mesh plant tissue in a platinum dish add 5 ml. of a saturated barium hydroxide solution, and place the dish in a cool muffle furnace. Determine the moisture content of a separate portion of the sample by drying it for 4 hr. at 100° C. Raise the temperature of the muffle furnace to 600° C. and ignite the dish for 2 hr. To the cold residue add 5 ml. of 70 per cent. acetic acid (10 ml. may be necessary with high boron contents), triturate the mixture with a rubber-tipped glass rod, and centrifuge the suspension in a 15-ml. centrifuge tube. Transfer 1 ml. of the clear supernatant liquid to a 125-ml. flask of boron-free glass and add 10 ml. of the chromotrope-B solution (*supra*), mix, allow to stand for colour development for at least 30 min., and adjust the temperature to that used in preparing the standard curve. Using 0·5-in. square cells, determine the percentage transmission at 620 mμ. against a reference solution prepared similarly but with 1 ml. of 70 per cent. acetic acid in place of the boron solution. A filter is not used when making the readings. Determine μg. of boron in the aliquot from the standard curve and calculate the total boron in the sample by means of the equation, boron p.p.m. (dry basis) = ab/w, where a is the number of millilitres of acetic acid added to the ash, b is the number of μg. of boron per ml., and w is the dry weight (g.) of the sample.

Silver, lead, and strontium (except in small amounts) interfere with the colour development. Organic anions interfere but are removed by the ignition. Nitrates, nitrites, chromates, and selenates react with the reagent to form a red compound, and fluorides prevent colour development by formation of borofluoride ion.

Since oven-drying causes loss of boron, the sample must be air-dried and the moisture determined separately. It is important that, for ignition, the sample with barium hydroxide should be placed in a cool furnace and that the temperature should then be raised to 600° C. The colour does not obey Beer's law strictly, but is reproducible with different batches of the reagent solution. In a sample of known boron content the recovery was 94·5 per cent.

A. O. Jones

Rapid Determination of Total Nitrogen in Ammonium Nitrate Fertiliser. R. D. Miller (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 373–381)—The method presented is the Spencer Chemical Company's Standard Procedure N-6 for determining total nitrogen in ammonium nitrate fertiliser. It is specific for ammonium salts and should not be confused with methods for mixed fertilisers. In the original method, of which this is a revision, the sample (about 1 g.) was dissolved in 100 ml. of water in a 250-ml. Erlenmeyer flask, 25 ml. of neutral 20 per cent. formaldehyde were added, the mixture was heated to 60° C., and, after cooling to 30° C., the liquid was titrated with 0·1 *N* sodium hydroxide until the end-point to phenolphthalein indicator (5 drops) was permanent for 30 sec.

Since descriptions of this method in the literature do not specify heating of the liquid after addition of formaldehyde, this detail was investigated and it was found that results with the same sample heated and unheated before titration agreed: also, that instead of titrating to the first pink colour remaining for 30 sec., titration until 1 drop of 0·25 *N* sodium hydroxide produced no perceptible change at the point of contact gave better and more consistent results. To use this end-point it is necessary to standardise the sodium hydroxide solution against pure ammonium nitrate to the same end-point, and it is believed that the pH at this end-point is that corresponding to the completion of the reaction.

For further evidence of the accuracy of the method it was compared with the Kjeldahl method and with a method depending upon the freezing-point of ammonium nitrate solutions. With the Kjeldahl method for comparison the results obtained by the two methods were in closer agreement than can normally be expected with two different Kjeldahl determinations with the same sample.

The apparatus for the "fog-point" method consists of a variable speed motor with a glass stirrer fixed to a solid base to prevent excessive vibration. From the same base a thermometer is suspended vertically and fastened rigidly just clear of the stirrer. A 250-ml. beaker holds the hot solution and just behind it a light is placed to permit close observation of the transparency of the liquid. The hot ammonium nitrate solution is poured into the beaker in such a manner that no crystals form on the sides. As the temperature drops and approaches the fog-point a scum forms on the surface of the liquid, and this serves to accelerate the process and to prevent supersaturation. The fog-point is the temperature at the point when the clear liquid becomes opaque. In order to check the fog-point method a graph was constructed from information from Seidell's *Solubilities of Inorganic and Organic Compounds in Water*, Vol. II, p. 103, 2nd Ed., and from authoritative information published by Rauich (U.S.S.R., 1933). The fog-point method proved trustworthy and superior to a specific gravity method previously used and was therefore considered suitable for comparison with the titration method. In comparison with this method the formaldehyde method showed good accuracy and excellent reproducibility.

Procedure—Weigh about 1·5 g. of the ammonium nitrate in a tared beaker, dissolve it in distilled water, and transfer the solution to a 250-ml. Erlenmeyer flask. Add 30 ml. of 20 per cent. formaldehyde of reagent grade and neutral to phenolphthalein and titrate with 0·25 N sodium hydroxide with phenolphthalein as indicator until the addition of 1 drop of the sodium hydroxide solution produces no perceptible colour change at the point of contact. If n ml. of 0·25 N sodium hydroxide are used in the titration and w is the weight of the sample, the percentage of nitrogen is given by $0{\cdot}025 \times 2{\cdot}802\, n/w$, in accordance with the equation

$$6HCHO + 4NH_4NO_3 + 4NaOH \rightarrow C_6H_{12}N_4 + 4NaNO_3 + 10H_2O.$$

The 0·25 N sodium hydroxide must be standardised against pure ammonium nitrate. A. O. Jones

Determination of the "Available" Magnesium engendered in Mixtures of Superphosphate with Olivine, Serpentine, Magnesite, and their Calcines. L. J. Hardin, W. H. MacIntire, and H. S. Johnson, jun. (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 405–419)—The present authors have demonstrated that adequate amounts of available magnesium are generated in moistened mixtures of concentrated superphosphate with olivine and serpentine, without diminution of available phosphoric acid.

The only official procedure for determining magnesium in mixed fertilisers is the one prescribed for acid-soluble content (*Methods of Analysis of the A.O.A.C.*, 6th Ed., 1945, **35**, 2.51, 2.53). In the present work a method is developed for determination of available magnesium in magnesium-fortified fertilisers, the term "available" being understood to include magnesium attributable to mono-, di-, and tri-magnesium phosphates, to magnesium ammonium phosphate and to the oxide, hydroxide, carbonate, and sulphate, and to exclude the magnesium of materials that release it only slightly or slowly.

The magnesium compounds added to triple superphosphate for experimental analyses were olivine, serpentine, and magnesite and the calcines of these. The reactions occurring in these mixtures are indicated by the equations

$$CaH_4(PO_4)_2.H_2O + MgSiO_3 + 3H_2O = CaHPO_4.2H_2O + MgHPO_4.3H_2O + SiO_2$$

and

$$CaH_4(PO_4)_2.H_2O + MgCO_3 + 3H_2O = CaHPO_4.2H_2O + MgHPO_4.3H_2O + CO_2$$

Procedure—Leach about 1 g. of a 50-mesh sample with 100 ml. of 2 per cent. citric acid solution at room temperature by successive decantations through a Shimer filter. Digest the residue and filter-pad with an additional 100 ml. of the reagent in a 250-ml. fertiliser flask for 30 min. at 50° C. by means of the end-over-end rotator in a constant-temperature apparatus (MacIntire *et al.*, *J. Assoc. Off. Agric. Chem.*, 1947, **30**, 161, Fig. 1). Filter by suction and wash the residue five times with 5-ml. portions of water at 65° C. Combine this with the liquid obtained by the original leaching in a 250-ml. fertiliser flask, dilute to volume, and mix. Transfer a 10-ml. or 25-ml. aliquot of this solution to a 400-ml. beaker, separate calcium by double precipitation as oxalate and determine the magnesium by the conventional magnesium ammonium phosphate procedure.

The following precautions are recommended. The aliquot should be such that the calcium content is low, but its magnesium content should be such as to ensure a final weight of 20 to 50 mg. of magnesium pyrophosphate. The initial precipitation of the calcium should be made by the oxalic acid and ammonium oxalate procedure. It is important that the precipitation be made in a solution of about 200 ml. containing the ammonium chloride from, at least, 10 ml. of concentrated hydrochloric acid. The second calcium precipitation should be made similarly from a solution of 150 to 175 ml. After filtration of the second precipitate of calcium oxalate the combined filtrates are evaporated with nitric acid and the residue is dissolved in diluted hydrochloric acid. Necessity for removal of iron and aluminium is obviated by addition of 2 to 3 g. of citric acid to the solution before precipitation of magnesium ammonium phosphate. When high accuracy is desired any appreciable amount of manganese should be removed. With ordinary fertiliser mixtures, the tedious removal of solvated silica was found to be unnecessary. In the evaporation of the filtrates before precipitation of magnesium ammonium phosphate, the solution should not be evaporated to dryness, but, if carbonisation of the citric acid occurs, re-clarification can be effected by additions of nitric acid followed by further digestion.

A. O. Jones

Organic

Determination of Phosphorus in Organic Compounds. A. R. Wreath (*J. Assoc. Off. Agric. Chem.*, 1948, **31**, 800)—The author recommends an acid digestion on some organic compounds instead of the semi-micro Parr-bomb method, because the former requires less apparatus and allows the use of a larger sample. The volumetric procedure of the Association of Official Agricultural Chemists is followed after neutralisation.

Procedure—Weigh 0·5 to 1·0 g. of sample, transfer to a 400-ml. beaker, add about 50 ml. of nitric acid (S.G. 1·42) and evaporate to 25 ml. Add from 15 to 20 ml. of 60 to 70 per cent. perchloric acid and evaporate to fuming. Cool, dilute to 250 ml., neutralise an aliquot, and complete the determination by the A.O.A.C. volumetric procedure (*Official and Tentative Methods of Anlysis*, 1945, p. 22).

Caution—If the solution becomes brown in colour during evaporation, remove from the source of heat at once and add a small quantity of nitric acid. Continue, but repeat the cooling and the addition of nitric acid if necessary. Failure to observe this precaution may result in an explosion.

A. H. A. Abbott

Colorimetric Determination of Caronamide. H. S. Collins and M. Finland (*J. Lab. Clin. Med.*, 1949, **34**, 509–515)—Caronamide (4_1-carboxyphenylmethanesulphonanilide) enhances and prolongs the levels of penicillin in body-fluids by inhibiting its renal excretion. A modified procedure is described for the colorimetric determination of caronamide in serum based on that of Ziegler and Sprague (*Ibid.*, 1948, **33**, 96–100; Abst., *Analyst*, 1948, **73**, 681): they used nickel - aluminium alloy in aqueous alkali to break down the caronamide to *p*-aminobenzoic acid which was then determined by established procedures. The duration of the reaction, the temperature at which it is carried out, the amount of catalyst, and the presence of serum were all found to influence markedly the degree of recovery of caronamide or *p*-aminobenzoic acid. The greatest yield was obtained when the reaction was carried out as in the procedure described below.

Procedure—Dilute 0·5 ml. of serum with 5 ml. of 5 per cent. sodium hydroxide, add 2 drops of *n*-octyl alcohol to minimise foaming, followed by 0·125 or 0·25 g. of the Raney catalyst. Allow to stand for 2 hr. at room temperature, then dilute to 50 ml., and filter through No. 42 Whatman paper. Add a 5-ml. aliquot to each of two matched tubes containing 0·5 ml. of 6 *N* hydrogen chloride; then add 0·5 ml. of 0·2 per cent. sodium nitrite solution to one tube and 0·5 ml. of water to the other, the latter serving as a blank. Allow diazotisation to proceed for 5 min. before adding 0·5 ml. of 2 per cent. ammonium sulphamate to both tubes and, after 3 min., 0·5 ml. of 0·1 per cent. N-(1-naphthyl)-ethylenediamine dihydrochloride also to each tube. Allow the colour to develop in a dark place for 30 min., then make the spectrophotometric readings, using a wavelength of 540 mμ.

A. H. Adams

Use of a Fluorescent Adsorbent for the Chromatography of Colourless Compounds. J. W. Sease (*J. Amer. Chem. Soc.*, 1947, **69**, 2242–2244)—Colourless compounds that absorb ultra-violet light can give chromatograms on a fluorescent adsorbent because the movement of the zones, on development by elution, can be observed as non-fluorescent areas when the column is illuminated with ultra-violet light. Adsorbents with fluorescent and adsorbent properties can be prepared by mixing 2·5 per cent. of a finely powdered, inorganic fluorescent substance, such as zinc sulphide, with a standard chromatographic adsorbent, such as alumina or silica gel.

Procedure—Pour 5 to 10 ml. of a solution, in light petroleum, of the two substances to be separated on to a column 1·8 cm. in diameter and 15 cm. in length. Develop the zones with additional light petroleum and observe their movement in a darkened room by means of ultra-violet light.

Extrude the column and cut out the portions of adsorbent containing a shadowed zone. Elute each zone with 30 to 40 ml. of 95 per cent. alcohol and determine the qualitative and quantitative composition of the resulting solutions.

To recover the adsorbent, elute thoroughly with alcohol, wash with light petroleum, and dry in the air at room temperature. Recovered material is slightly less adsorbent than the unused mixture.

A. H. A. Abbott

Use of Silver Vanadates as Absorbents for Sulphur Dioxide. G. Ingram (*Analyt. Chim. Acta*, 1949, **3**, 137–143)—Silver vanadates produced under various conditions show different absorptive powers towards sulphur dioxide in a combustion train. To prepare an active product that, when dry, absorbs sulphur dioxide at room temperature, and does not absorb carbon dioxide, heat an intimate mixture of 2·31 g. of silver oxide and 2·07 g. of silver metavanadate for 24 hr. at 150° C. A fine powder is more efficient than granules of size 10 to 14 mesh, and shows a sharper change from green to brown when it is spent. Products prepared as above, but without heating, absorb some carbon dioxide in addition to the sulphur dioxide.

M. E. Dalziel

Determination of Aromatics in Petroleum Fractions. Separation from High-Boiling Fractions by Adsorption. M. R. Lipkin, W. A. Hoffecker, C. C. Martin, and R. E. Ledley (*Anal. Chem.*, 1948, **20**, 130–143)—The method, which is an adaptation of the Mair and Forziati procedure

(*J. Res. Nat. Bur. Standards*, **1944, 32,** 165), involves dilution of the sample with an equal volume of pentane, percolation through a fixed amount of silica gel, washing the gel with pentane until the refractive index of the percolate drops to that of pentane, and desorption of the aromatics with a mixture of benzene and methyl alcohol. With the refractive index as an indication of the cut-point, two fractions are collected, the first, washed through with pentane, containing the paraffins, naphthenes and non-aromatic olefines, and the second, obtained by desorption with benzene and methyl alcohol, containing all the aromatics and aromatic olefines and most of the non-hydrocarbons. Since this procedure is used with fractions boiling above 200° C., it is relatively easy to distil the solvents from the separated fractions by using a stripper that has been specially designed for the work. Quantitative recovery of the separated hydrocarbons is effected and the fractions are available for further testing.

Apparatus and reagents—The gel column consists of a glass tube of inside diameter 5 cm. and length 120 cm. with a stopcock at its lower end. The receiver, usually a litre graduated cylinder, is immediately underneath the stopcock. A refractometer capable of giving refractive indices reproducible to 0·0001 or 0·0002 is necessary. In the investigation a special flow-through refractometer was placed in series between the column and the receiver so that changes in the composition of the effluent could be followed.

The stripping apparatus consists of a 500-ml. separating funnel leading to a 250-ml. round-bottomed distilling flask with its side-arm leading downwards into a 500-ml. flat-bottomed flask immersed in crushed ice. The distilling flask is fitted also with a capillary tube reaching to within a short distance of the bottom of the flask and serving for the introduction of a stream of nitrogen during the distillation. The distillation flask is immersed in a water-bath heated by a 1-litre hemispherical Glas-col heater. Spherical joints permit disconnection of the side-arm tubing, the 500-ml. separating funnel and the round-bottomed flask.

The adsorptive capacity of the silica gel (28- to 100-mesh) should be such that by percolation of 20 ml. of a standard mixture of 20 per cent. of benzene in methyl*cyclo*hexane through 10 g. of gel in a column of 10 mm. diameter no less than 5 ml. of filtrate free from aromatics (as determined by refractive index of small portions) will be obtained. The solvents used are pentane (*i.e.*, any mixture of pentanes boiling between 30° and 40° C.), benzene and methyl alcohol. These should be free from high-boiling residues, since their presence would make it difficult or impossible to separate the solvent completely from the concentrate by distillation.

Procedure—Weigh 100 ± 1 ml. of the sample with an accuracy of 0·05 g. and dilute it with 100 ml. of pentane. Tamp a small wad of glass-wool into the bottom of the glass column and then tamp 1 kg. of the silica gel into the column, avoiding the formation of channels. Introduce 500 ml. of pentane into the column and open the stopcock. When the pentane has just disappeared into the column, add the diluted sample and rinse the container with small portions of pentane. As the liquid level falls to the level of the gel, add successively to the column two 500-ml. portions of pentane and as many 200-ml. portions as are necessary to displace the non-aromatic hydrocarbon fraction from the gel (*infra*), and collect the percolate in the graduated cylinder. Regulate the flow to 7 ± 1 ml. per min.

Collect approximately 1-ml. samples at specified intervals for the measurement of refractive index, returning any sample left after this determination to the receiver. Make the refractive index measurements after 100 and 1000 ml. of percolate have been collected and at 100-ml. intervals thereafter, until the refractive index is within 0·0002 of the first refractive index measured, *i.e.*, the refractive index of the pentane itself. When this point is reached add no more pentane to the column, but start desorption of the aromatics with a mixture of 400 ml. of benzene plus 100 ml. of methyl alcohol. Add two 500-ml. portions of methanol later to complete the desorption.

Two interfaces appear as the desorption proceeds down the column, the lower interface indicating the position of the benzene front and the more pronounced upper one the position of the methanol. When the lower interface is three-fourths of the way down the column, change the receiver and begin to collect the aromatic fraction of the percolate. The volume of the first fraction is usually from 1600 to 2000 ml. The rate of flow of the percolate may decrease during the collection of the second fraction even with the stopcock fully open. While the column is draining, the stripping apparatus is made ready.

Strip the solvents from the two fractions separately. Pour 500 ml. of the fraction into the separating funnel and allow it to drain into the tared round-bottomed flask until the latter is half filled. Close the stopcock, heat the water-bath gradually and admit a small stream of nitrogen through the capillary tube to prevent bumping. When the solvent begins to distil open the stopcock and allow the sample to run into the flask at the rate at which the solvent is removed. If the second fraction contains "fines" from the silica gel it must be filtered into the separating funnel, the filter being washed with pentane. If the original sample has an initial boiling-point near 200 ml., maintain the temperature of the water-bath below 85° C. to minimise losses. With lube oil samples maintain the temperature of the bath above 95° C. As the separating funnel empties refill it until the sample is exhausted, rinsing in the final portions with pentane. When drops of solvent cease to form at the end of the delivery tube into the cooled receiver, disconnect the flask containing the stripped sample from the funnel and receiver and heat it in the water-bath for another hour, then weigh it, and repeat the procedure until its weight is constant to within 0·1 g.

The weight of the non-aromatic fraction plus that of the aromatic fraction should equal at least 98 per cent. of the sample charged; otherwise the determination must be repeated. If w_1 and w_2 are the weights of the aromatic fraction and the non-aromatic fraction, respectively, and d_1 and d_2 are the corresponding densities of the fractions, the percentage by volume of aromatic hydrocarbons in the sample is given by

$$100\,(w_1/d_1)/(w_1/d_1 + w_2/d_2)$$

With highly olefinic samples it may be necessary to use more than 2000 ml. of pentane to cause the refractive index of the percolate to reach that of pentane. With some very unusual samples the capacity of the gel may be exceeded and the refractive index of the percolate may begin to rise without reaching the refractive index of pentane. The minimum value reached should then be used as the cut-point and the receiver should be changed to collect the aromatic fraction. A check determination should be made with 80 ml. of sample instead of 100 ml. and, if the results differ, the second is the correct one. A brownish colour in the first percolate indicates excessive impurity in the gel and another batch should then be used.

Separation of the desorbent by distillation yields better results than the use of such desorbents as *iso*propyl alcohol, carbitol, and 2-methyl-2 : 4-pentanediol, which can be separated from the oil by extraction with water. Although methyl alcohol is a good desorbent, easily separated from the aromatics by distillation in the gas oil and lube oil ranges, it is not completely miscible with these aromatics, and the mixture of benzene and methyl alcohol is more satisfactory.

Application of the method to samples of known composition showed that its accuracy and precision are both within 1 per cent. The method classifies all hydrocarbons containing one or more aromatic rings as aromatics, whether the side-chain is paraffin, olefine, naphthene ring or *cyclo*-olefine.

A. O. Jones

Shark Liver Oil from Trinidad. F. Major and R. W. Pearman (*Bull. Imp. Inst.*, 1948, **46**, 53–56)—Nine samples of shark liver oil from Trinidad were examined. Eight had been extracted with makeshift equipment and the ninth represented residual sludge that had settled from the oil. All had a distinct fishy odour and deposited stearin on cooling. The oils were filtered at 40° C. and were then subjected to chemical examination with the following results: moisture (in the unfiltered oil), 0·1 to 2·0 per cent.; sp.gr. (40°/40° C.), 0·9179 to 0·9218; n^{40}, 1·4698 to 1·4740; sap. val. (mg. of KOH per g.), 181·4 to 188·7; free fatty acids (as oleic acid), 0·9 to 4·1 per cent., excluding one sample containing 37·5 per cent.; unsaponifiable matter, 1·3 to 5·4 per cent.; iodine value (Wijs, 30 min.), 132·5 to 175·2; vitamin A (I.U. per g.), 4340 to 50,500. The sample of sludge contained also approximately 1·2 per cent. of waxy material.

The high content of free fatty acid and the presence of stearin indicate that the oils are crude, but with improved equipment, and digestion of the fresh livers with alkali, satisfactorily neutral oil should be obtainable.

A. O. Jones

Determination of Monomer in Polystyrene. J. J. McGovern, J. M. Grim, and W. C. Teach (*Anal. Chem.*, 1948, **20**, 312–314)—Two methods, a spectrophotometric and a solubility method, have been devised for the determination of monomer in polystyrene.

Spectrophotometric method—Monomeric styrene possesses absorption bands at 282 and 291 mμ., whilst polystyrene shows only slight general absorption in these regions. Dimeric and similar polystyrenes of low molecular weight would interfere with a determination based on absorption in these regions, but such material is absent, or nearly so, from polymerised styrene; determinations of mixtures of styrene with polystyrenes of various molecular weights showed no dependence on the latter.

Procedure—Prepare a calibration curve by measuring the optical densities at 282 and 291 mμ. (the authors used a Beckman instrument, model DU, and the optical densities were read directly from the instrument) of known monomer - polymer mixtures dissolved in chloroform. The use of both wavelengths is not essential but is a convenient method of replication of the determination. Divide the optical densities obtained by the total sample concentrations and plot the quotient so obtained against the percentage of monomer. A straight line should be obtained but, owing to the slight general absorption by the polymer, it will not pass through the origin. Determine the optical densities of chloroform solutions of known concentration of the samples to be determined and obtain the content of monomeric styrene as a percentage from the calibration curves.

Solubility method—Polystyrene is insoluble in methanol, whilst the monomer, dimer, and trimer are soluble. As the last two do not occur in appreciable quantities in polystyrene, the methanol-soluble portion may be used to determine the monomer content.

Procedure—Dissolve 10 g. of polystyrene in 100 ml. of toluene. Mix slowly with 700 ml. of methanol (this can be done in a Waring Blendor, the toluene solution being added to methanol already in the Blendor). Filter the methanol slurry of precipitated polystyrene, rinse with methanol, and dry at 65° C. until constant in weight. The difference between the initial and final weights represents the amount of monomer present. Any additives present are, by this method, included as monomeric styrene.

Comparison of the two methods—Agreement of the order of ±0·2 for monomer contents up to 13 per cent. is obtained between the two methods and this represents the reproducibility of the solubility method. The amount of additive present was successfully determined as the difference between the apparent styrene content given by the solubility method and the styrene content determined spectrophotometrically.

W. C. Wake

Potentiometric Titration of Fatty Acids with Silver Nitrate. O. Harva and P. Ekwall (*Acta Chem. Scandinavica*, 1948, **2**, 713–726)—Potentiometric titrations can be carried out on a number of saturated fatty acids by taking advantage of the low solubility of their silver salts. The potential difference between a silvered silver-wire indicator electrode and a normal calomel reference electrode immersed in a solution of the sodium salt of the fatty acid is plotted against the volume of 0·05 *N* or 0·1 *N* silver nitrate added, and the end-point is shown as an inflection on the curve.

Individual saturated fatty acids from C_{10} to C_{18}, and also oleic acid, can be determined by this method. Both components of binary mixtures of saturated fatty acids can be determined if the numbers of carbon atoms in the two acids differ by more than two; in addition, both lauric and capric acids can be determined in a mixture of these two acids. All three components of a ternary mixture can be determined if the mixture does not contain successive even acids between C_{14} and C_{18}.

J. G. WALLER

Inorganic

Qualitative Separation on a Micro-Scale. Analysis of the Gold Group of A. A. Noyes and W. C. Bray. O. König, W. R. Crowell, and A. A. Benedetti-Pichler (*Mikrochem.*, 1948, **33**, 281–297)—The scheme of Noyes and Bray (*A System of Qualitative Analysis for the Rare Elements*, New York, 1927) is converted to the micro-scale. New forms of capillary pipettes (Figs. 1 and 2) are used in which the intake tubes are of 1-mm. bore and the bulbs are of 0·5- to 2-ml. capacity. Figs. 3 to 5 show an extraction apparatus with its mode of operation.

The amount of each metal present is estimated by comparison of the centrifuged bulk of its precipitate with that obtained from known amounts.

PROCEDURE—*Preparation of samples*—If the starting material consists of the freshly precipitated metals, heat it for 10 to 15 min. with 0·5 ml. of 2 *M* nitric acid, which dissolves the mercury, most of the palladium, and some platinum. Centrifuge, evaporate the centrifugate to dryness, and re-dissolve it in 0·2 *M* hydrochloric acid. Dissolve the residue in the centrifuge tube in *aqua regia*, evaporate to dryness, evaporate with hydrochloric acid, re-dissolve in 0·2 *M* hydrochloric acid, and combine with the centrifugate. Treat any undissolved residue with hydrochloric and nitric acid mixture in a pressure bulb as described for alloy analysis (*infra*) and add it to the main solution. The total final volume of solution should be about 0·3 ml.

Gold—Heat the solution in boiling water and add sodium nitrite crystals in slight excess. After 10 min., the gold separates as a turbidity or as a mirror. Centrifuge, and wash twice with 0·1 ml. of water, reserving the centrifugate and washings. To the precipitate add 1 drop of hydrochloric acid and 1 drop of nitric acid, heat in boiling water and dry at 140° C. Cool, moisten with 1 drop of hydrochloric acid, dry at 140° C., and dissolve in 1 drop of water. Add 2 drops of a 10 per cent. solution of tetraethylammonium chloride, and allow to stand for 1 hr. Centrifuge the yellow crystalline precipitate of the gold compound. To confirm the presence of gold, wash the precipitate with alcohol and dry it at 140° C. Dissolve in 1 drop of hydrochloric acid and 1 drop of nitric acid in a water-bath, and evaporate to dryness at 140° C. Add 1 drop of hydrochloric acid and dry again. Dissolve the residue in 1 drop of water and treat on a

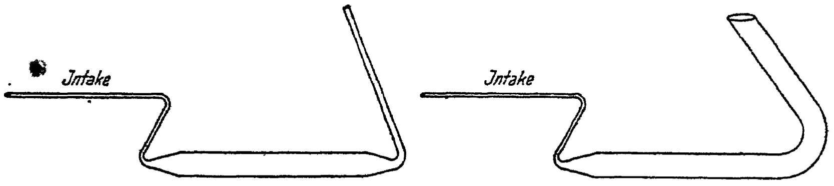


Fig. 1 Fig. 2

Capillary Pipette

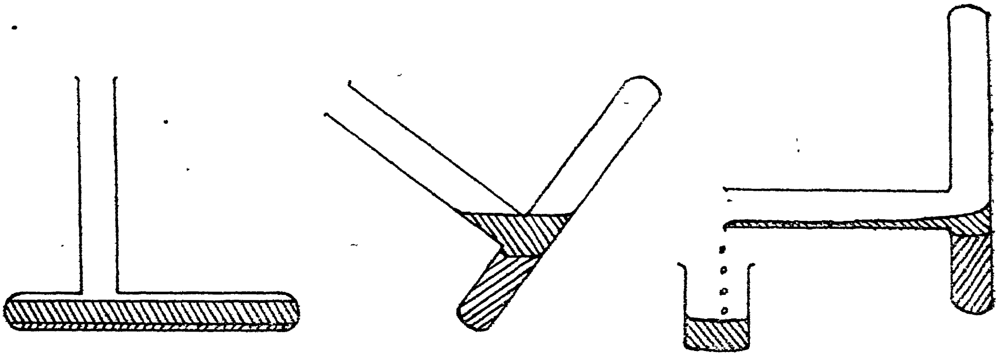


Fig. 3 Fig. 4 Fig. 5

Extraction Tube

microscope slide with a crystal of hexamine. Yellow branched needles or rectangular plates are obtained.

Mercury—To the centrifugate from the gold precipitation add 2 to 3 drops of a freshly prepared 0·1 per cent. solution of Reinecke's salt in 0·2 per cent. hydrochloric acid. Mercury forms a pink amorphous precipitate. Allow to stand for 10 min., and centrifuge.

Platinum—Transfer the centrifugate from the mercury precipitation to a 5-ml. beaker and evaporate it repeatedly with 0·5-ml. quantities of *aqua regia*, and then twice with 0·5-ml. quantities of hydrochloric acid. Dissolve the residue in 0·3 ml. of water, transfer to a cone, and heat in a water-bath for 5 min. with addition of 2 drops of a 10 per cent. solution of sodium bromate. Add 2 per cent. sodium hydrogen carbonate solution until a violet reaction is obtained with cresol red and continue heating with stirring for 15 min., testing the pH each 5 min. and re-adjusting with sodium hydrogen carbonate solution if necessary. Centrifuge, wash the precipitate twice with 0·1-ml. portions of water, combine the washings with the centrifugate in a micro-beaker, add 3 to 5 drops of hydrochloric acid, and evaporate to dryness at 125° C. Dissolve the residue in 5 drops of water, transfer to a cone, add 0·5 ml. of a 10 per cent. solution of phenylbenzyldimethylammonium chloride, and allow to stand for 5 min. Centrifuge the crystalline precipitate. Confirm the identity by heating the cone in boiling water. The platinum complex remains undissolved, whilst any palladium complex that may have separated from concentrated solutions will redissolve on heating. *Alternatively*, the platinum can be estimated as follows. Acidify the aqueous platinum solution with 1 drop of hydrochloric acid, dilute with a few drops of water, heat in boiling water, and treat with a rapid stream of hydrogen sulphide. Repeat the heating and hydrogen sulphide treatment three times, stopper the cone, and allow to stand for 15 min.; centrifuge. Confirm as follows. Dissolve the washed precipitate in 1 drop of hydrochloric acid and 1 drop of nitric acid, evaporate at 140° C., cool, add 1 drop of hydrochloric acid and repeat the evaporation. Dissolve the residue in 1 drop of water, transfer to a slide, and add a crystal of hexamine. Platinum is indicated by the appearance of small colourless octahedra.

Palladium—The precipitate obtained in the bromate - hydrogen carbonate treatment (*supra*) consists of the oxides of palladium, iridium, and rhodium. Dissolve this precipitate by heating in a water-bath with 1 drop of hydrochloric acid and 1 drop of nitric acid, and evaporate to dryness at 140° C. Dissolve the residue in 0·5 ml. of water, add 1 drop of hydrochloric acid and sufficient dimethylglyoxime in 1 per cent. alcoholic solution to precipitate the palladium; avoid a large excess. Allow to stand 1 hr., centrifuge, wash the precipitate twice with water, and mix the washings with the centrifugate. The amount of palladium can be estimated from the volume of the precipitate but the sulphide is more suitable. Dissolve the precipitate by heating with 2 drops of *aqua regia* and evaporate at 125° C. Repeat the evaporation after adding 2 drops of hydrochloric acid. Dissolve in 0·5 ml. of water, add 1 drop of hydrochloric acid and precipitate the sulphide as directed for platinum. Palladium can be confirmed by the yellow colour it gives with thioglycollic acid.

Rhodium—Warm the filtrate from the palladium dimethyglyoxime, to remove the alcohol, and evaporate repeatedly with *aqua regia* and then with hydrochloric acid. *Method A*—Dissolve the residue in 3 drops of water and transfer to a cone. Nearly saturate with potassium nitrite, add 6 drops of ethanol, mix, and allow to stand for 6 hr.; centrifuge. To confirm, evaporate the rhodonitrite repeatedly with 3 drops of *aqua regia* and then with 1 drop of hydrochloric acid and a crystal of potassium chlorate. Dissolve the residue in 2 drops of water. (*a*) Treat a drop of this solution on a slide with a crystal of hexamine hydrochloride; after 5 to 10 min., magenta prisms are observed under the microscope. Any iridium present changes the colour to brown. (*b*) Boil a portion of the solution with 1 drop of 20 per cent. dithioglycollic acid; a yellow colour develops slowly on cooling. (*c*) Heat 1 to 2 drops of the solution with 1 drop of a 40 per cent. solution of stannous chloride in 30 per cent. hydrochloric acid; a raspberry red colour develops. *Method B*—Dissolve the residue in 0·5 ml. of 3 *M* hydrochloric acid, transfer to a cone, and heat in a water-bath. Add an excess of solid potassium ethyl xanthate and continue heating for 30 min. with stirring; centrifuge. To confirm, evaporate the precipitate with 3 drops of hydrochloric acid and a few crystals of potassium chlorate and take up the residue in 2 drops of water. Use this solution in the confirmatory tests given under method A (*supra*).

Iridium—*Method A*—Evaporate the filtrate from the rhodonitrite to remove alcohol and then evaporate it repeatedly with 3 drops of *aqua regia* and then with 1 drop of hydrochloric acid. Dissolve the residue in 0·3 ml. of hydrochloric acid and add 0·1 ml. of saturated bromine water. Heat in a water-bath, then boil, and finally evaporate at 125° C. Dissolve the residue in 0·3 ml. of water, add 0·03 ml. of bromine water, heat for 5 min. in a water-bath while adding 2 per cent. sodium hydrogen carbonate solution until a violet colour is obtained with cresol red. Continue heating 30 min. with stirring, and centrifuge. *Method B*—To the filtrate from the rhodium ethyl xanthate add 3 drops of hydrochloric acid and a few crystals of potassium chlorate, and evaporate to dryness. Re-dissolve in 0·3 ml. of hydrochloric acid and 0·03 ml. of bromine water. The rest of the procedure is the same as in method A. To confirm, wash the precipitate twice with water and dissolve it in 1 drop of hydrochloric acid and 1 drop of nitric acid in a water-bath. Evaporate at 140° C., add 1 drop of hydrochloric acid to the cooled residue, and evaporate again. Dissolve in 2 drops of water, transfer to a slide and add a crystal of "Helmitol" (formamol). Tiny ruby-red octahedra or crosses indicate presence of iridium.

ALLOYS—*Preparation of solutions*—Alloys containing iridium, but not mercury, are dissolved by the following method. Prepare a glass pressure-bulb of 2-mm. wall-thickness, 6 mm. inside diameter, and 30 mm. length. Seal on to the top a capillary of 2-mm. wall thickness, 2-mm. bore, and 30-mm. length. Introduce the sample and 0·5 ml. of hydrochloric acid. Make a capillary 25 mm. long and thin enough to pass into the bulb; close it at one end, fill it with nitric acid and push it into the bulb, closed end first. Seal the bulb, then mix the contents. Imbed the bulb in sand and heat for 24 hr. in an oven at 140° C. Before opening, cool in a "dry ice" - acetone mixture. Evaporate the solution to dryness at 125° C., evaporate the residue repeatedly with hydrochloric acid, and dissolve in 0·2 *M* hydrochloric acid. If osmium or ruthenium is present drain the contents of the bulb into hydrochloric acid saturated with sulphur dioxide.

Extraction of gold with ethyl acetate—This method can be used when mercury is absent. Evaporate the solution of chlorides to dryness and take up the residue with 0·3 ml. of hydrochloric acid. Shake the solution with two 0·3-ml. portions of ethyl acetate. Wash the extracts with 0·1 ml. of 3 *M* hydrochloric acid, and evaporate at 125° C. Evaporate the residue repeatedly with 3 drops of *aqua regia* and then with 1 drop of hydrochloric acid. Dissolve in a drop of water and precipitate with tetraethylammonium chloride.

Precipitation of gold with sulphur dioxide—Dissolve the chloride residue from the original solution in 0·1 *M* hydrochloric acid and add 0·3 ml. of saturated sulphur dioxide solution. Heat in a steam-bath for 15 min., add more sulphur dioxide solution, and heat again for 5 min. Centrifuge the precipitated gold and wash it with 6 *M* hydrochloric acid.

When perchloric acid is used for the separation of ruthenium tetroxide by distillation, no interference results in the procedures described, except that phenylbenzyldimethylammonium chloride gives a white precipitate with the perchlorate ions, and platinum must therefore be precipitated as sulphide.

W. C. JOHNSON

Reduction of Ferricyanides and a Sensitive Test for the Detection of Hydrogen Peroxide. M. Kohn (*Analyt. Chim. Acta*, 1949, 3, 38–40)—In the presence of zinc ions and sodium acetate, yellow zinc ferricyanide is reduced on warming with hydrogen peroxide to the white ferrocyanide. The analagous reaction with copper ferricyanide to form brown copper ferrocyanide occurs rapidly at room temperature; in the absence of sodium acetate, reaction is slower.

Reagent—Mix 21 ml. of 0·1 *M* copper sulphate, 10 ml. of 0·1 *M* potassium ferricyanide, 6 ml. of 15 per cent. sodium acetate solution, and 20 ml. of water; store in a dark bottle in a cool, dark place.

Procedure—Dilute 2 or 3 drops of the reagent with 10 ml. of water, thus producing a yellow suspension, and add enough calcium carbonate to give a small sediment. Shake well and divide into two parts; to one part add the test solution and compare it with the other as "blank" after about 0·5 hr. The test is sensitive to 2·5 μg. of hydrogen peroxide in 1 ml.

For drop-reaction tests, mix 1·5 ml. of the reagent with 0·5 g. of calcium carbonate and spot the suspension on paper. The yellow spot turns brown in the presence of hydrogen peroxide. If traces of hydrogen peroxide are to be detected, keep the test paper in the dark for 15 min. Under these conditions, the test is sensitive to 0·5 μg. in one drop.

Other reducing compounds must be absent.

M. E. DALZIEL

Diphenylbenzidine as a Reagent for Vanadium. J. Hoste (*Analyt. Chim. Acta*, 1949, **3**, 36–37)—Diphenylbenzidine can be used to detect vanadium as vanadate at a concentration of 1 part in 10^6; the test is specific for vanadate in absence of other oxidising agents. The following ions do not interfere: silver, mercury, copper, lead, bismuth, cadmium, arsenic, antimony, tin, selenium, tellurium, germanium, molybdenum,^{VI} tungsten,^{VI} niobium, tantalum, aluminium, chromium, uranyl, neodymium, cerous, zirconium, thorium, beryllium, thallium, gallium, indium, manganese, cobalt, nickel, barium, strontium, calcium, magnesium, lithium, sodium, potassium, rubidium, caesium, and ammonium, unless their concentration is such that a masking colour is produced, *e.g.*, the concentration of chromium, titanium, cobalt, or nickel must not exceed 1 part in 1000 if 1 part of vanadium in 10^6 is to be detected. Ferric iron must be removed as the fluoride complex.

Procedure—To 5 drops of a saturated solution of diphenylbenzidine in glacial acetic acid and 1 drop of 10 per cent. aqueous potassium fluoride solution (if iron is present) on a spot plate add 1 drop of the almost neutral test solution. A green precipitate indicates vanadium at concentrations exceeding 1 part in 100,000, and a yellow colour at greater dilution.

M. E. DALZIEL

Polarographic Method for Copper, Lead, and Iron, using a Pyrophosphate Background Solution. C. A. Reynolds and L. B. Rogers (*Anal. Chem.*, 1949, **21**, 176–178)—The polarographic reduction waves for cupric, plumbous, and ferric ions, with 0·1 *M* sodium pyrophosphate as base electrolyte, are suitable for the determination of these metals in alloys. As the half-wave potentials for lead and iron are close to each other (−0·69 v. and −0·82 v. *versus* the saturated calomel electrode, respectively) one of these metals must be determined by another method if both are present in the sample. The reduction of cupric ions gives rise to reduction waves at −0·40 v. and −1·33 v. *versus* the saturated calomel electrode, and neither wave is close enough to the lead or iron wave to cause interference.

Procedure for brass samples—Dissolve 0·1 g. of the sample in 1 ml. of concentrated nitric acid, expel the oxides of nitrogen by boiling, and dilute to 250 ml. in a volumetric flask with 0·1 *M* sodium pyrophosphate solution. Mix 50 ml. of this solution with 1 ml. of a 2 per cent. solution of gum ghatti, add 1 g. of sodium sulphite, and examine polarographically.

Aluminium-, zinc-, beryllium-, and magnesium-base alloys can be examined in the same way, the aluminium- and zinc-base alloys being dissolved by adding 2 ml. of 5 *N* hydrochloric acid followed by 0·5 ml. of concentrated nitric acid.

J. G. Waller

Occurrence of Vanadium, Chromium, and Other Unusual Elements in Certain Coals. F. M. Reynolds (*J. Soc. Chem. Ind.*, 1948, **67**, 341–345)—There are indications that, should the need arise, certain elements could be obtained from the waste products of coal, *e.g.*, flue dust and coal ash in which enrichment occurs under some conditions. Thus, Morgan and Davies (*Chem. and Ind.*, 1937, **15**, 717) showed that flue dust from producer systems is often rich in germanium and gallium and that these metals can be recovered from this source. Enrichment to a less extent also occurs in coal ash.

Some occurrences in the coals of North Wales and Staffordshire are now reported, firstly the occurrence of vanadium, chromium, titanium, and nickel in a special type of coal and then some occurrences of copper, boron, lead, germanium, and strontium, which are of less importance. The occurrence of barium in coals of these areas has already been reported (Reynolds, *J. Soc. Chem. Ind.*, 1939, **58**, 64).

In the coalfields of Staffordshire and North Wales, bands of a vitrain-like coal occur, and are richer in vanadium, chromium, titanium, and nickel than are the coal seams of these areas, and though relatively small these deposits are widely distributed.

In the peacock seam of North Staffordshire, vitrain is distributed in some quantity in the stratum immediately above the seam, and associated with it over a wide area. Samples were coarsely crushed, and clean fractions were separated by flotation on a mixture of benzene and carbon tetrachloride of sp.gr. 1·35. The clean fractions were then crushed to pass a 72 B.S. sieve and thin layers were slowly heated to 800° C. in a good oxidising atmosphere, and that temperature was then maintained until the material was completely ashed. The ash was of an unusual yellowish-brown colour and had a tendency to sinter. In three samples the vanadium content (as V_2O_5) of the ash ranged from 10·1 to 14·1 per cent., and the chromium content (as Cr_2O_3) from 0·5 to 1·0 per cent. In two of the samples the titanium contents (as TiO_2) were 9·2 and 15·0 per cent. and the nickel contents (as NiO) 9·2 and 11·2. These figures are abnormally high since coal ash usually contains less than 2 per cent. of titanium and only traces of vanadium, chromium, and nickel. Only the ash of the vitrain from the roof showed this abnormality, and no unusual elements were found either in the other portions of the seam or in the true vitrain of the seam.

Other deposits of vitrain similar to the one described were found associated with a number of seams in the North Staffordshire and North Wales coalfields, and the analyses suggest that high contents of chromium, vanadium, and nickel are general in these occurrences. In seven samples from these two coalfields, fractionated and ashed in the manner described, the chromium content ranged from 0·1 to 1·6 per cent., the titanium from 5·2 to 29·5 per cent., the nickel from a trace to 3·0 per cent., the vanadium from 1·3 to 14 per cent., and five of these seven samples contained more than 5 per cent. of vanadium in the ash. The ash contents of these vitrains ranged from 1·6 to 3 per cent., in marked contrast with the very low ash contents of certain cauldron vitrains of Northumberland and Durham. Spectrographic examination of two of these vitrain ashes confirmed the presence of boron, chromium, nickel, and vanadium with heavy traces of antimony, barium, caesium, copper, germanium, lead, molybdenum, phosphorus, rubidium, strontium, and zinc, and traces of arsenic, beryllium, cobalt, indium (in one ash only), lanthanum, manganese, silver, and tin.

Fragments of vitrain from the roof of a Northumberland seam were found to be similar in every respect to the vanadium-rich vitrains of North Staffordshire and North Wales. Cauldron vitrain from Northumberland and Durham is also vanadium-bearing, and the amounts of chromium and vanadium present in the ash are of the same order as in the ash of the Peacock seam vitrain, but the ash of the latter is 15 times that of the former.

Vanadium-bearing vitrain also occurs outside coal measures, *e.g.*, occasional lenticles and bands occur near Gillow Heath in Staffordshire, interbedded with shale in strata of Millstone Grit age. Whitby jet, a black, lustrous substance occurring in isolated lumps in shale and thus resembling the vitrain deposits described and the cauldron vitrains of Northumberland and Durham, also is rich in vanadium.

On the basis of a spectrographic examination, samples of coal ash from Staffordshire and North Wales were selected for the quantitative determination of boron, lead, germanium, and strontium with these results—boron, 0·24 per cent.; lead, 0·44 per cent.; germanium (as GeO_2), 0·1 per cent.; strontium was detected chemically in barytes from the Quaker seam in North Wales, the amount being estimated as not less than 1 per cent. of the mineral.

To determine vanadium and chromium the coal ash was fused for 1 hr. with sodium carbonate and a small amount of potassium nitrate and the melt was extracted with hot water containing some alcohol (to reduce any manganese present). Chromium was determined by matching the solution against standard potassium dichromate solutions, the extract being concentrated if necessary. To determine vanadium the extract was then acidified with sulphuric acid, the vanadium was oxidised to the quinquevalent state with permanganate and was precipitated with cupferron. The cupferride was ignited, then re-dissolved by pyrosulphate fusion and the vanadium was finally determined by reduction to the quadrivalent state with sulphur dioxide and titration with standard permanganate solution. In the complete analysis of samples of ash it was necessary to guard against possible interference by vanadium in the determination of iron and titanium and phosphorus. When required, iron and titanium were separated from vanadium by precipitation as hydroxides with an excess of sodium hydroxide solution and phosphorus was separated from vanadium by precipitation of the latter with cupferron. Nickel was determined as the dimethylglyoxime complex following its precipitation as sulphide in the filtrate from the ammonia group.

Examination of the vertical distribution of copper in one seam showed that copper was present in every part of the pillar section, but the greater part was in the top 11 in. of the seam. The highest percentage found was 6 per cent. in the vitrain, but not more than 10 per cent. of the seam consisted of vitrain. This does not indicate a good industrial source of copper. A. O. JONES

Separation of Calcium from Magnesium. I. Investigation of the Oxalate Method by Means of Radioactive Indicators. V. P. Shvedov (*J. Anal. Chem. Russ.*, 1948, **3**, 147–152)—The separation of calcium and magnesium has been studied by normal means and with artificial radioactive isotopes of calcium. The oxalate precipitate always contains appreciable amounts of magnesium even when its weight is approximately theoretical. This precipitate cannot be freed from magnesium by re-precipitation.

A target of metallic calcium was bombarded with rapid deuterons for 30 min., the preparation was dissolved in hydrochloric acid, calcium precipitated as carbonate, followed by re-precipitation, and the product used for giving standards containing 0·0001 to 0·0050 g. of calcium. The samples had a half-life period of between 3 hr. 36 min. and 4 hr. 6 min., and hence contained mainly the 2·5-hr. calcium isotope.

Precipitation was carried out as follows: To 150 to 200 ml. of a solution containing different proportions of calcium and magnesium, but not more than 100 mg. of each, were added a few drops of methyl orange, 5 ml. of concentrated hydrochloric acid, and then 50 ml. of hot 5 per cent. ammonium oxalate solution. The solution was heated to 75° C., and then slowly treated with 12 per cent. aqueous ammonia solution, with stirring, until the reaction was alkaline. After 1 hr. the solution was filtered, the precipitate of oxalate and carbonate washed 5 times with cold 1 per cent. ammonium oxalate solution, then dried and ignited, transformed to sulphate, and weighed. Experiments showed that the amount of co-precipitated magnesium depended on the precipitation temperature (thus, when this amount was 1·6 per cent. at 20° C. it became 3·5 per cent. at 100° C.), but 75° C. was kept as the standard temperature. Magnesium was precipitated from the filtrate as phosphate. By means of Geiger counters the radioactivity of the magnesium precipitate was determined and the calcium content found from a calibration curve. The amount of magnesium co-precipitated with the calcium was found by difference from the activity of the original calcium in the control and test samples and in the phosphate precipitate.

Gravimetric determinations showed that with a Ca to Mg ratio of 10 : 1 the apparent error was about 1 part in 100 for the calcium content, whilst with a Ca : Mg ratio of 1 : 10 the error was 4 to 15 parts in 100. Double and triple precipitations reduced an error of 15 parts in 100 to a nearly constant 7 parts in 100. Thus, with 0·0212 g. of calcium and 0·212 g. of magnesium the weights of the second and third oxalate precipitates were high by the equivalents of 0·0017 and 0·0018 g. of calcium.

Radioactive tests showed that with 0·1 g. each of calcium and magnesium the amount of magnesium in the first calcium precipitate varied between 2 and 4·8 per cent. of the total weight, and the amount of calcium in the magnesium precipitate between 0·5 and 5·2 per cent. of the weight of calcium taken. With 0·1 g. of calcium and 0·01 g. of magnesium, the corresponding errors were 1·2 and 1 per cent., respectively, and with 0·01 g. of calcium and 0·1 g. of magnesium they were 20 to 40 per cent. and 24 to 55 per cent. G. S. SMITH

Separation of Calcium from Magnesium. II. Sulphate Method. V. P. Shvedov (*J. Anal. Chem., U.S.S.R.*, 1948, **3**, 290–294)—In aqueous acetone medium calcium sulphate coagulates more quickly than in alcoholic medium, whilst magnesium sulphate tends to precipitate more quickly from aqueous alcoholic solutions than from aqueous acetone solutions. Tests on the separation of calcium and magnesium were made gravimetrically and by the use of a radioactive isotope of calcium (*cf.* abstract above). Precipitation was carried out in solutions containing 2 ml. of concentrated sulphuric acid and 60 to 80 ml. of acetone in 100 ml., and the precipitates were washed with acetone-water mixtures of the same relative proportions as in the solution. With calcium alone, variations of the acetone : water ratio between 4 : 1 and 3 : 2 and in the time of standing at room temperature between 1 and 3 hr. had no effect on the results. The apparent calcium content, as determined gravimetrically, of mixtures of calcium and magnesium taken in ratios between 10 : 1 and 1 : 10 showed little variation, but the activity tests showed that even with the ratio 10 : 1 several per cent. of the amount of calcium taken remained in solution. The method, therefore, gives correct results because of a compensation of errors. It can be recommended for general use as an improvement on the method of precipitating in aqueous alcoholic solution.

G. S. SMITH

Volumetric Determination of Molybdenum in Steel. P. Ya. Yakovlev (*Zavod. Lab.*, 1948, **14** 397–398)—Molybdenum can be determined by extracting the molybdenum from a thiocyanate solution with ether and titrating the molybdenum with permanganate after reduction with zinc amalgam.

Procedure—Dissolve 1 g. of steel in 50 ml. of hydrochloric acid, or, if necessary, in *aqua regia*. Treat with nitric acid and evaporate twice with hydrochloric acid. Add to the residue 10 to 15 ml. of concentrated hydrochloric acid, heat to dissolve the salts, cool, add 50 ml. of 20 per cent. stannous chloride solution and 30 to 50 ml. of 7 per cent. potassium or ammonium thiocyanate solution. Extract with 20 to 25 ml. of ether in a separating funnel, and then with two further portions of 10 to 15 ml. In a clean beaker collect the ether extracts and washings, mix with 30 to 40 ml. of water, and boil off the ether. Add carefully to the aqueous solution so obtained 15 ml. of concentrated sulphuric acid in several portions, and then nitric acid until the solution is clear. Evaporate to fumes. If the solution becomes black, add more nitric acid and evaporate again. Dilute the cooled liquid to 50 ml., transfer it to a Someya reduction apparatus, reduce with zinc amalgam, and titrate with potassium permanganate in the presence of carbon dioxide.

The method is suitable for steels containing vanadium, titanium, and aluminium.

Results for steels containing from 2·6 to 7·1 per cent. of molybdenum agreed closely with those obtained by an accurate colorimetric method.

G. S. Smith

Rapid Method of Analysis of Tungsten and Molybdenum Alloys. V. G. Goryushina and T. V. Cherkashina (*Zavod. Lab.*, 1948, **14**, 873–874) —The use of hydrofluoric acid or the need for fusion in the analysis of tungsten alloys can be avoided by using Gusev's solvent, a saturated solution of oxalic acid containing perhydrol. Furthermore, the excess of oxidising agent is removable by simple boiling, and the application of a reductometric finish is simplified.

Procedure for tungsten and molybdeno-tungsten alloys—Treat 0·2 g. of the finely-divided sample with 20 ml. of oxalic acid solution saturated in the cold, and 2 to 3 ml. of perhydrol, heat gently to complete dissolution, and then boil off the excess of perhydrol. In alloys containing no iron add a known amount, *e.g.*, 5 ml. of 0·1 N tervalent iron to assist the decomposition of the peroxide. Transfer to a titration flask containing 75 ml. of diluted hydrochloric acid solution (2 + 1), and titrate potentiometrically with 0·1 N chromous chloride solution.

The method was tested on ferro-tungsten containing 73·2 per cent. of tungsten (found W 73·6 and 73·6 per cent.), ferro-molybdenum containing 58·8 per cent. of molybdenum (found Mo 58·8 and 58·8 per cent.) and molybdeno-tungsten alloy containing 80 per cent. of molybdenum (found Mo 80 and 79·7 per cent.). G. S. Smith

Determination of Graphite in Malleable Cast Iron. V. M. Shpeyzman and E. V. Elenevskaya (*Zavod. Lab.*, 1948, **14**, 879–880)—Results almost as accurate as gravimetric determinations of graphite can be made by measurements of graphite areas on a polished section of cast iron. The work is simplified by using a scale of circles with diameters from 1 to 10 mm. by 0·5-mm. increments, which can be applied to a 100-magnification image of the metal surface on opal glass. A number of experiments showed that accurate results could be guaranteed if not less than four or five places were selected at random for measurement, and that more were superfluous.

G. S. Smith

Determination of Sodium in Clays by means of an Automatic Apparatus. P. D. Korzh (*Zavod. Lab.* 1948, **14**, 1088–1091)—In the arc, admixture of calcium sulphate with complex silicates containing sodium, *e.g.*, clays, etc., causes sodium to be set free and continuous passage of sodium through the arc occurs. The spectra obtained show that while sodium is being vaporised the lines of the other elements present are absent, and that as soon as the sodium lines disappear those of calcium appear. The disappearance of the sodium lines occurs always at the same voltage of the arc. With the appearance of calcium and other lines the voltage suddenly increases and it is possible to find the exact moment of the disappearance of the sodium lines by observation of a voltmeter in the arc circuit. The arc may therefore be used for determining sodium in clays without spectroscopic apparatus merely by recording the time elapsed before the sudden increase in voltage. Magnesium sulphate is found to be a better diluent than calcium sulphate. An automatic apparatus is described and illustrated.

In tests, 20 mg. of material were placed in a hollow of a carbon electrode (positive), and a 220-volt, 3-amp. D.C. arc was used for excitation with a gap between the electrodes of 2 mm. The voltmeter was read every 10 sec., and the sodium doublet 5688·3 to 5682·6 A. observed in the spectrum. With pure salts the voltage remained constant during the time of observation 60 sec., sodium sulphate giving 21 v., sodium silicate 24 v., calcium sulphate 29 v., and magnesium sulphate 48 v. With 1 : 1·5 mixtures of clay and calcium sulphate and of clay and magnesium sulphate, the initial voltage agreed with that for pure sodium silicate. When the sodium lines disappeared, after about 40 sec., the voltage rose to 30 in the former case, and to 41, and thence to 48, in the latter.

With the automatic apparatus many samples of mixtures of clays and magnesium sulphate are stated to have been examined giving results [not recorded in the paper] of good reproducibility and high accuracy. G. S. Smith

Reviews

INDUSTRIAL RHEOLOGY AND RHEOLOGICAL STRUCTURES. By HENRY GREEN. Pp. vii + 311. New York: John Wiley & Sons Inc. London: Chapman & Hall, Ltd. 1949. Price 44s. net.

Henry Green was one of the pioneers of the science of rheology, and worked with E. C. Bingham long before the latter and his colleagues founded the first Society of Rheology or even chose the name of the science.

After the early twenties, however, Green published little in the way of rheology until 1940. From this latter date until the time of his death his work on thixotropy with his colleague Miss Ruth Weltmann, and also his measurements of "tack," formed the subject of many contributions to rheological literature.

It is difficult to decide for what type of reader this posthumous book was really intended; even though at the start of Chapter VII the author specifically tells us that ". . . the ultimate desire is to introduce rheology into industry where it is needed. The immediate problem is to induce Bill (as Scott Blair calls the investigator) to become an industrial rheologist." (In passing be it said that "Bill" was the original craftsman, not the investigator.)

The admirable appendixes addressed to "the laboratory investigator" and "the laboratory director" show an all too rare appreciation of practical needs. And yet it must regretfully be admitted that the rest of the book is unlikely to be of any great help to the young physicist, or more often chemist, with whom the author rightly had so deep a sympathy.

There are two main reasons for this: first—and the author has made some attempts to meet this criticism (p. 86)—almost the whole of the book, apart from a very elementary treatment of general rheology, deals with those phenomena and problems with which Green himself worked, and he often gives little except his own treatment of them. Even the bibliography of the work of other rheologists becomes very thin during the period 1925 to 1940. This would be no disadvantage if the book were intended for highly specialised rheologists, but this is clearly not so.

Secondly, and much more serious, the book contains far too many inaccurate and misleading statements and definitions. The discerning reader will, of course, "know what the author means" but, to the beginner, these inaccuracies can only lead to confusion.

Space allows the mention of only a few:—

p. 50–51. "In the capillary tube viscometer the material under test is extruded and, therefore, cannot be measured in its broken down state" (but the direction of shear can be reversed).

p. 80. "Reiner's claim is that all real bodies can be pictured as combinations of these three elementary models (Hookean, Newtonian and St. Venant bodies)."

p. 289 [part of definition of an elastic deformation]. "When the stress is removed the displaced molecules return to the position they occupied before the stress was applied. As a consequence, in elastic deformation, strain is proportional to stress" (*cf.* non-linear elastic systems obeying Bach's law).

p. 291. "Laminar flow . . . the opposite of turbulent flow."

p. 295. "Specific viscosity: see relative viscosity. These terms are interchangeable." (In fact, $\eta_{sp} = \eta_{rel}^{-1}$.)

"Stress: the force that causes strain" (no comment needed!).

Elsewhere there is much that is muddled. For example, the dangers of relying on "apparent viscosities" are rightly pointed out (p. 19), but the definition in the glossary (p. 286) is confusing: "An imaginary quantity . . . the viscosity the non-Newtonian would have (at a given rate of shear) if it were a Newtonian. It is obtained experimentally by dividing the shear stress by the rate of shear, and then multiplying by the instrumental constant."

Yet, in spite of these defects, to the careful reader there is much that is interesting in the book, not least (and this was perhaps Green's greatest contribution to rheology) his insistence on the advantages of looking and seeing for oneself. That the mechanisms underlying quite a number of rheological phenomena can readily be observed under the microscope is a fact all too often forgotten.

Much of the book is a description of Green and Weltmann's work on thixotropy which has already been criticised by fellow-rheologists. His experiments on the measurement of "tack" in ink are interesting, but the use of the tackmeter whose "original object was to analyse pull resistance and express it in rheological terms" presents a very great over-simplification even if the author were right in claiming that "from the inkman's point of view, *tack is pull resistance*" (p. 113). Just how complex the matter really is may be seen from the discussions in this country reported in *Nature*, 1942, **150**, 159 and in *Paint Technology*, 1944, **9**, 211.

Yet, in spite of these criticisms, which must be made, the book contains much that is of interest to the careful reader; and to those of us who did not have the pleasure of knowing the author personally it reveals something of a character that must have been altogether likeable. There is a directness and charm about the style which makes Green's work very readable and, if the student must be advised to read the book warily, he will never for a moment find his task dry or dull.

G. W. SCOTT BLAIR

ERRATA:

VOL. 74, 1949:

p. 351. At the end of the first item in the list of references, *for* "540," *read* "54."

p. 362. Equation (iv), line 14 from the bottom of the page, should *read* $W = \frac{ap}{T_2} - bD$.

p. 454. Please *add* the following acknowledgment in the final paragraph—"Our thanks are due to our colleague Miss Nora Baxter for providing spectroscopic data."

p. 479. In the 7th line below the figure, *for* "between the two slides," *read* "between the two solids."

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